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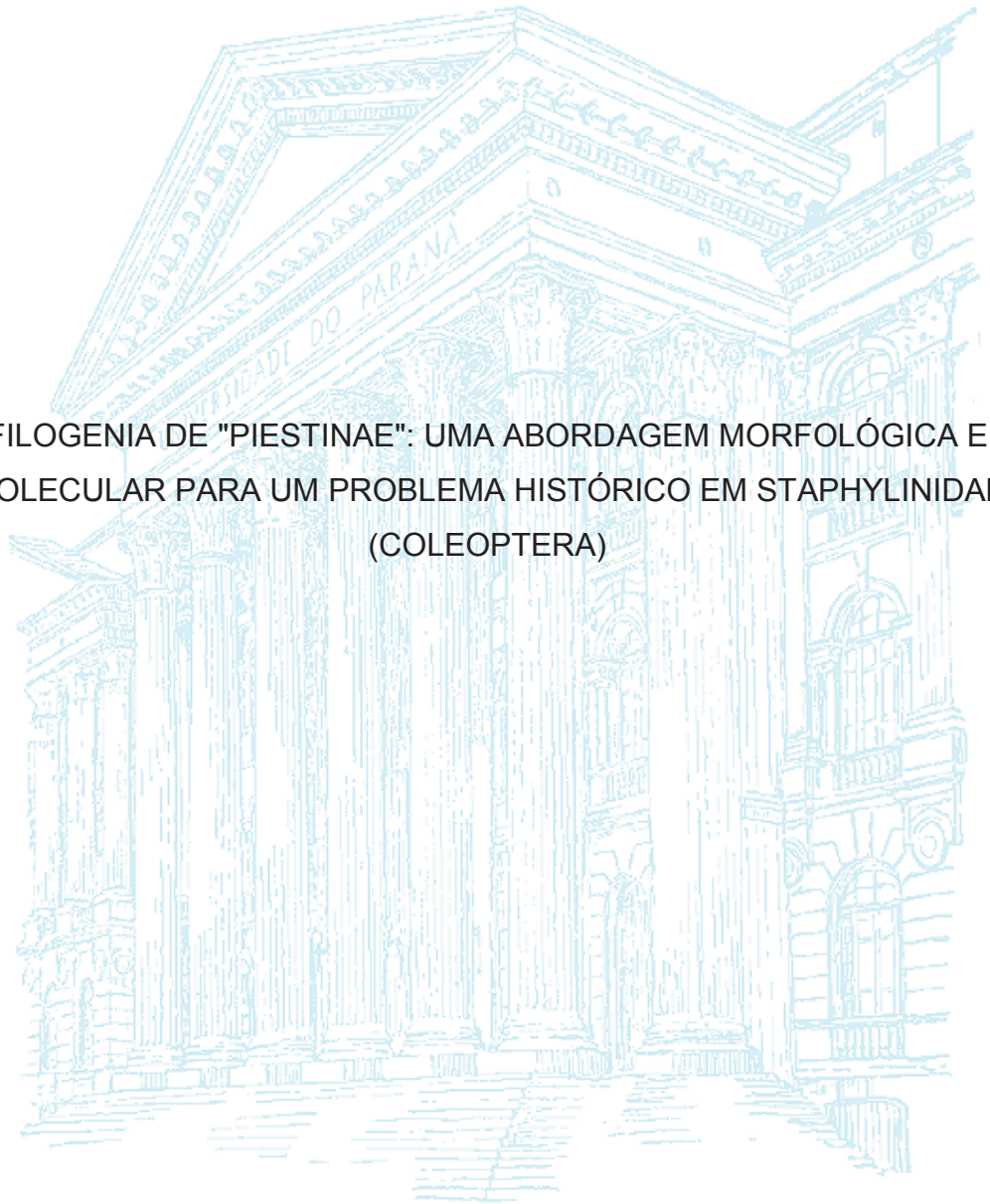
SIDNEI BORTOLUZZI DA SILVA



FILOGENIA DE "PIESTINAE": UMA ABORDAGEM MORFOLÓGICA E MOLECULAR PARA UM PROBLEMA HISTÓRICO EM STAPHYLINIDAE (COLEOPTERA)

CURITIBA

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Entomologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências Biológicas.

Orientador: Prof. Dr. Edilson Caron
Co-orientador: Dr. Leonardo Foti

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS BIOLÓGICAS (ENTOMOLOGIA) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **SIDNEI BORTOLUZZI DA SILVA** intitulada: **Filogenia de "Piestinae": uma abordagem morfológica e molecular para um problema histórico em Staphylinidae (Coleoptera)**, sob orientação do Prof. Dr. EDILSON CARON, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua Aprovação no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 19 de Fevereiro de 2020.

EDILSON CARON
Presidente da Banca Examinadora (UNIVERSIDADE FEDERAL DO PARANÁ)

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“A man's mind stretched to a new idea
never goes back to its original dimensions.”

Oliver Wendell Holmes, Sr.

RESUMO

Piestinae Erichson, 1839 é uma pequena subfamília de Staphylinidae historicamente definida por caracteres homoplásticos e inicialmente utilizada como repositório para uma variedade de besouros estafilinídeos que não se encaixavam em outros lugares. Mesmo com trabalhos recentes sobre classificação, análise filogenética e descrição de espécies, fósseis ou atuais, essa subfamília não é considerada monofilética. O principal objetivo do presente estudo foi reavaliar a monofilia da subfamília sob diferentes fontes de informação. Nossas análises foram baseadas em 164 caracteres morfológicos de adultos, 74 caracteres morfológicos de larvas e dados de sequência de DNA de três regiões gênicas (28S: 1636pb, CO1: 844pb e Wg: 451pb). As relações filogenéticas foram inferidas usando inferência bayesiana e máxima parcimônia. A monofilia de Piestinae foi estabelecida por dados morfológicos, porém, este mesmo resultado não foi recuperado por dados moleculares. O relacionamento entre os clados na base da subfamília permanece duvidoso, contudo, dois principais clados foram identificados: *Piestus* + (*Hypotelus* + *Eupiestus*) e "*Siagonium*" + *Piestoneus*. O gênero *Siagonium* foi recuperado como parafilético em relação ao *Piestoneus*, porém, para confirmar esse resultado e efetuar as alterações taxonômicas serão necessárias análises com uma mais amostragem de espécies do gênero *Siagonium* mais abrangente.

Palavras-chave: estafilinídeos, Grupo Oxyteline, piestíneos, sistemática filogenética.

ABSTRACT

Piestinae Erichson, 1839 is a small subfamily of Staphylinidae that is historically defined by plesiomorphic characters and was earlier used as a repository for a diverse assortment of rove beetles that did not fit well elsewhere. Even with recent works about the classification, phylogenetic analysis and description of species, fossils or recent, this subfamily is considered to be non-monophyletic. The main aim of the present study was to re-evaluate the monophyly of the subfamily under three different approaches. Our analyses were based on 164 morphological characters from adults, 74 morphological characters from larvae and DNA sequence data from three gene regions (28S: 1636pb. CO1: 844pb, Wg: 541pb). Phylogenetic relationships were inferred using Bayesian inference and maximum parsimony. The monophyly of Piestinae was established by morphological data and was not recovered by molecular data. The relationship between the clades at the basis of the subfamily is remained doubtful, but two main clades were identified: *Piestus* + (*Hypotelus* + *Eupiestus*) and "*Siagonium*" + *Piestoneus*. The genus *Siagonium* was recovered as paraphyletic with respect to *Piestoneus*, however, more comprehensive analysis with a broad number of species of the genus is necessary to confirm this result and make the taxonomic changes.

Keywords: Oxyteline Group, phylogenetic systematics, piestines beetles, rove beetles.

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SUMMARY

1. INTRODUCTION

1.1. Staphylinidae

Staphylinidae Latreille, 1802, or rove beetles, includes over 63,000 species in 3,870 genera, placed into 35 extant subfamilies, representing one of the greatest Metazoan's radiations (Grebennikov & Newton 2009; Ahn *et al*. 2017; Żyla & Solodovnikov 2019) in number of described species, as well as, occupying a tremendous diversity of habitats, microhabitats and feeding habits (see Thayer 2016 for details).

They are well adapted to most heterogeneous habitats distributed on all continents and major islands, except Antarctica (Thayer 2016). Overall, they are predators in leaf litter and decaying plant matter, also exploiting several types of microhabitats such as fungi, invertebrates and vertebrates nests, seacoasts, with some staphylinids are considered to feed on ectoparasites of small mammals or parasitoids of fly puparia (see Thayer 2016 for details of microhabitats).

Staphylinids exhibit tremendous variation in form, but the vast majority can be distinguished from other beetles by their combination of short truncate elytra exposing more than half of the flexible abdomen, six or occasionally seven visible abdominal segments, and (with few exceptions) contiguous procoxae (Newton *et al*. 2000).

Even after removal of the oldest Staphylinidae reported fossil – *Leehermania prorova* Chatzimanolis *et al*., 2012 –, with dubious placement in any of extant subfamilies, to any extinct lineage within the beetle suborder Myxophaga, there is no doubt that staphylinids occur by the Middle Jurassic Period (ca. 170 Mya) (Cai *et al*. 2012; Chatzimanolis *et al*. 2012; Mckenna *et al*. 2015b; Zhang *et al*. 2018). According with recent works on evolutionary history of the beetles lineage (Zhang *et al*. 2018; Toussaint *et al*. 2017), the Staphylinidae lineage would arose in the Middle Jurassic (ca.170 Mya) and that many lineages of the family began your divergence in the Late Jurassic.

The constitution and the relationship between the clades in Staphylinidae, as well as the delimitation of your taxonomic units, have been discussed over the years and remain controversial. (e.g. Ganglbauer 1895; Crowson 1955; Coiffait 1972; Lawrence & Newton 1982, 1995; Naomi 1985; Newton & Thayer 1988, 1995; Hansen

1997b; Caterino *et al*. 2005; Lawrence *et al*. 2011; Thayer 2016). The family Silphidae Latreille, 1807 – currently with nearly 190 described species, mostly associated with and/or feeding on carrion (Sikes 2005) – composes the Staphylinid Group of Lawrence & Newton (1982) along with Staphylinidae. Some recent authors consider the family as a derived lineage from the Staphylinidae clade (e.g. Lawrence & Newton 1982; Newton & Thayer 1995; Hansen 1997b; Grebennikov & Newton 2009 – molecular data only; Mckenna *et al*. 2015a) or a sister group to Staphylinidae clade (Grebennikov & Newton 2009; Grebennikov & Newton 2012).

Lawrence & Newton (1982) summarized the classification of Coleoptera and proposed the 22-odd staphylinid subfamilies recognized then could be organized into four main lineages or informal groups: the Omaliine Group, the Tachyporine Group, the Oxyteline Group and the Staphylinine Group.

Later studies about Staphylinidae classification, systematics and/or phylogeny were conducted to understand the evolutionary history of these four main lineages, e.g.:

- Newton & Thayer (1992) formally recognized Scaphidiinae Latreille, 1807 as a staphylinid subfamily; Apateticinae Fauvel, 1895 and Trigonurinae Reiche, 1865 were recognized as subfamilies, and formally named Empelinae and Solieriinae as staphylinid subfamilies;
- Ashe & Newton (1993) and Ashe (2005) focused on the phylogeny of the Tachyporine Group, although they did not test its monophyly properly;
- Newton & Thayer (1995) focused on the phylogeny of the Omaliine Group, ranking the former Pselaphidae Latreille, 1802 down to a subfamily nested well within a monophyletic Omaliine Group, and corroborated the placement of Microsilphinae Crowson, 1950, Glypholomatinae Jeannel, 1962, Micropeplinae Leach, 1815 and Dasycerinae Reitter, 1887 in this group;
- Grebennikov & Newton (2009) focused on the phylogeny of the Staphylinine Group, ranking the former Scydmaenidae Leach, 1815 down to a subfamily nested well within a monophyletic Staphylinine Group;
- Grebennikov & Newton (2012) focused on the phylogeny of the Oxyteline Group, found Apateticinae Fauvel, 1895 and Trigonurinae Reiche, 1865 falling at the base of Staphylinidae, recognized Oxyteline Group in a more restricted

sense formed by Scaphidiinae, Oxytelinae Fleming, 1821, Osoriinae Erichson, 1839 and Piestinae Erichson, 1839 (a non-monophyletic subfamily); and

- Żyla & Solodovnikov 2019 provided a multilocus molecular phylogeny of the subfamily Staphylininae, reclassifying it. Both subfamilies Xantholininae and Platyprosopinae were revised status, besides the new subfamily Coomaninae was established.
- The Staphylinid Group and their relationship are summarized in Fig 1.

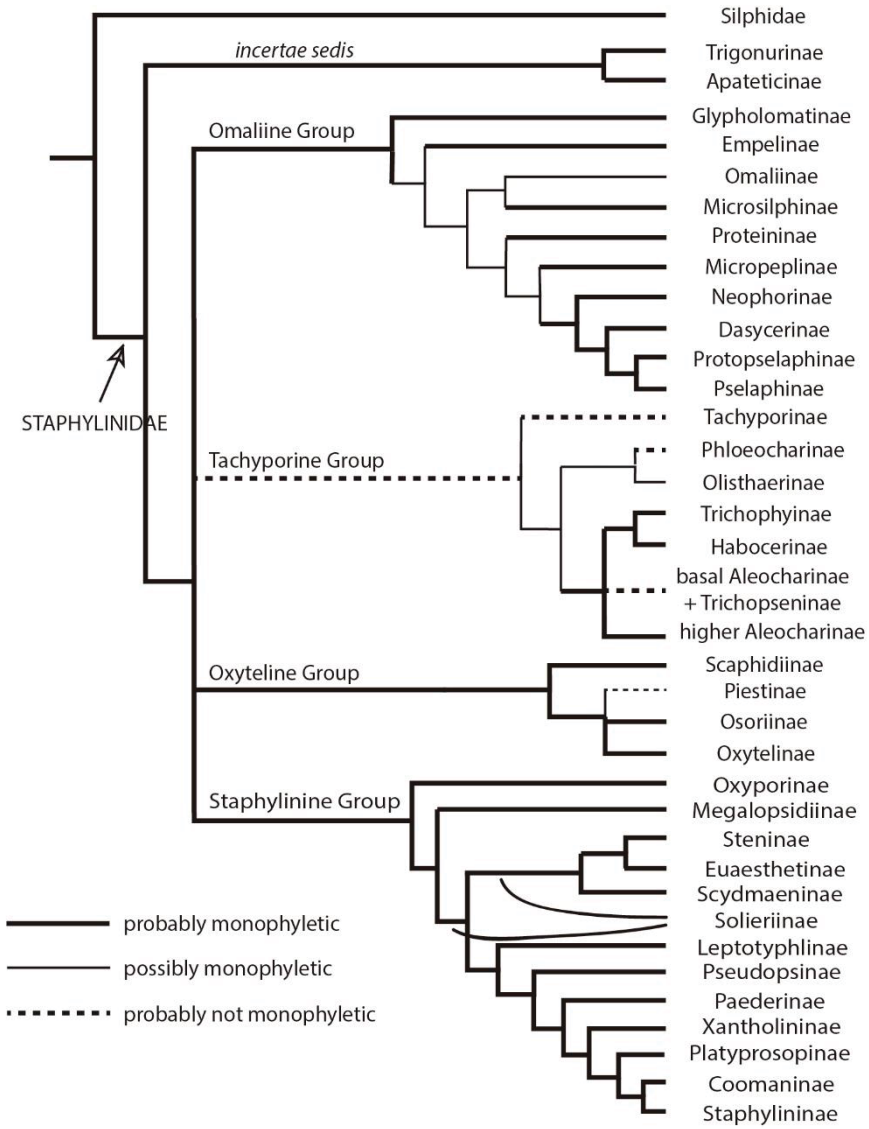

Figure 1. A phylogenetic schema for the Staphylinid Group, based largely on Thayer (2005), Ashe (2005), Grebennikov & Newton (2009; 2012) and Żyla & Solodovnikov (2019).

The Staphylinid Group is supported by morphological characters of both adults and larvae (Lawrence & Newton 1982; Newton & Thayer 1995; Beutel & Molenda 1997; Hansen 1997b; Grebennikov & Newton 2012; Thayer 2016) and by analyses of small sampling of molecular sequence data (Ballard *et al*. 1998; Mckenna *et al*. 2015a). However, none of the four major proposed staphylinid lineages/groups was recovered as monophyletic in recent well-sampled molecular or morphological phylogenetic studies (e.g. Hansen 1997b; Ballard *et al*. 1998; Beutel & Leschen 2005; Caterino *et al*. 2005; Mckenna *et al*. 2015a).

1.2. Oxyteline Group

Earlier composed of six subfamilies: Apateticinae, Trigonurinae, Oxytelinae, Piestinae, Osoriinae and Scaphidiinae (e.g., *sensu* Thayer 2005), the Oxyteline Group is the smallest of the four putative lineages, composed of 5,943 described species and about 215 genera, currently grouped into four subfamilies now (Fig. 2) (Grebennikov & Newton 2012).

The species are small to moderate in size (1–10 mm long) and variable in shape, have short to long elytra, found in diverse habitats but often associated with decaying trees or other decaying matter. They are biologically exceptional within Staphylinidae in being, as far as known, entirely saprophagous or (Scaphidiinae) mycophagous rather than carnivorous like the great majority of species in the other three staphylinid groups (Lawrence & Newton 1982, 1995; Grebennikov & Newton 2012; Thayer 2016). Adults and larvae have an elongated and looped gut, unusual in comparision to other staphylinids (Mckenna *et al*. 2015a).

Recent phylogenetic study (Grebennikov & Newton 2012) clarified the basal position of the Oxyteline Group inside Staphylinidae, showing that the evolution of the family probably were derived throughout transition on the alimentary behavior from the omnivorous/saprophagous groups to carnivorous groups (although reversal and parallelism are found in some cases). Besides, the analyses in this paper consistently identified a monophylum of Scaphidiinae + (“Piestinae” + Osoriinae + Oxytelinae), which the authors kept the name “Oxyteline Group” but in a slightly restricted sense, (*i.e.*, without Apateticinae and Trigonurinae which were recovered at the base of

Staphylinidae, outside of the Oxyteline Group (Fig.2)). However, they did not include characters of the gut in their analysis, as pointed out by Mckenna *et al*. (2015a).

Other phylogenetic studies have been conducted on or within the larger subfamilies. Oxytelinae was demonstrated as monophyletic by Herman (1970), notably by their common possession of a unique pair of defensive glands at the abdominal apex, and phylogenetic relationships among the oxyteline tribes and genera were initiated in that study and partially extended by Newton (1982b) and Makranczy (2006). The monophyly and internal relationships among tribes and genera of Scaphidiinae were explored by Leschen & Löbl (1995). The monophyly of Osoriinae has not been previously tested but it was supported in Grebennikov & Newton (2012) although the relationships among the sampled exemplar taxa had suggested that the tribe Thoracophorini may not be monophyletic, in disaccording with the study of this tribe by Irmler (2010).

Piestinae has not been demonstrated as monophyletic (e.g., Thayer 2005; Grebennikov & Newton 2012; Mckenna *et al*. 2015a), even after removal of the tribes Apateticini and Trigonurini as separate subfamilies by Newton & Thayer (1992) based on larval characters indicated in Newton (1982b), see below.

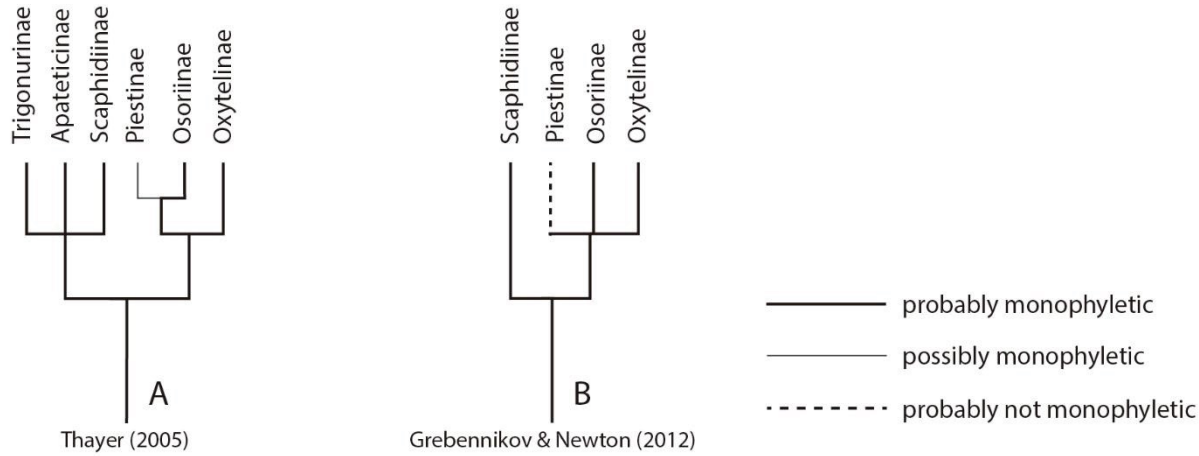

Figure 2. Hypotheses of the composition of the Oxyteline Group. (A) Sensu Thayer (2005); (B) Sensu Grebennikov & Newton (2012).

1.3. The problem called Piestinae

Piestinae Erichson, 1839 is one of the oldest of the 35 currently recognized extant staphylinid subfamilies. It is a small group comprising 118 species into ten genera (seven extant, three extinct) (Yamamoto *et al*. 2018) (see Table 1). The distribution of Piestinae is significantly sparse in the western Palearctic and Africa (Yamamoto *et al*. 2018) and five out of seven extant genera have the southern hemispherical affinities (Neotropical and Austral regions) (Table 1).

All piestine taxa are found under bark and in rotten woods, or occasionally in leaf litter (Caron *et al*. 2012). The detailed biology is unknown, but they are nearly all saprophagous, and few could be mycophagous feeding habits (Thayer 2016). According to Crowson & Ellis (1969), some piestines possess tiny invaginations on mandibles similar to scotyline and cucujoid beetles with which may function as the fungal spore-transmitting mycangia.

Table 1. Overview of the genera of the subfamily Piestinae currently recognized in the world.

Genus	N species (118 spp.)	Geographical distribution
Eupiestus Kraatz, 1859	23	eastern Palearctic and Oriental regions
Hypotelus Erichson, 1839	13	Nearctic (Florida) and Neotropical regions
Parasiagonum Steel, 1950	1	New Zealand
Prognathoides Steel, 1950	1	Australia
Piestoneus Sharp, 1889	4	eastern Palearctic region
Piestus Gravenhorst, 1806	47	Nearctic (Florida) and Neotropical regions
Siagonium Kirby & Spence, 1815	25 (†1 sp.)	Holarctic and northern Neotropical regions
†*Paleosiagonium* Yue *et al.*, 2016	2	Chaomidian, China
†*Eopiestus* Cai & Lü, 2017	1	Kaliningrad, Russia
†*Propiestus* Yamamoto *et al*., 2018	1	Kachin, Myanmar

† – extinct piestine genus/species.

The subfamily is historically defined by plesiomophic characters and was earlier used as a repository for a diverse assortment of rove beetles that do not fit well elsewhere (Caron *et al*. 2012). However, several recent studies gradually refined the concept of the piestine rove beetles (e.g., Caron *et al*. 2012; Grebennikov & Newton 2012; Bortoluzzi *et al*. 2017). Since it is now much less heterogeneous, the monophyly

of the Subfamily is uncertain; and they appear to represent merely the part of the Piestinae + Osoriinae clade lacking the apomorphies of Osoriinae (Thayer 2016)

About the taxonomic changes into the subfamily, Bernhauer & Schubert (1910), for example, included nearly 30 genera in their concept of the group (as the tribe Piestini, included in the subfamily Oxytelinae). Gradually, many of those genera have been removed to form groups of their own or added to other well-defined subfamilies: Aleocharinae, Apateticinae, Micropeplinae, Osoriinae, Ploeocharinae and Trigonurinae (e.g., Blackwelder 1942; Herman 1972; Newton 1982a, 1982b, 1988; Newton & Thayer 1992, as examples) (see Herman 2001 for complete catalog).

Regards to phylogenetic knowledge, Thayer (2005) noted that Piestinae, in that current sense, is possibly a monophyletic group and a sister group of Osoriinae (Fig. 1), although cladistic analyses have been showing that the monophyly of the whole subfamily is not established yet (e.g., Grebennikov & Newton 2012; McKenna *et al*. 2015a), with Piestinae being paraphyletic with respect to Osoriinae and Oxytelinae.

Results by Grebennikov & Newton (2012), based on 240 parsimony-informative larval and adult morphological characters, indicate that the piestines are included in the clade comprised of the restricted sense of the Oxyteline Group and they formed a basal grade with respect of Osoriinae. Similar results were obtained by McKenna *et al*. (2015a) in the DNA-based approach with two molecular markers (28S and CAD) conduced based on 3430pb in totally. In their analyses, Piestinae was appeared to be paraphyletic (Bayesian analysis) or polyphyletic (maximum likelihood analysis) with respect to the subfamilies Oxytelinae and Osoriinae combined.

Cladistics analyses within subfamily Piestinae were performed only for the genera *Piestus* Gravenhorst, 1806 and *Hypotelus* Erichson, 1839 (e.g., Caron *et al*. 2012; Bortoluzzi *et al.* 2017), but none of these studies was focused on testing the monophyly of the subfamily. Caron *et al.* (2012) included as outgroup taxa representatives of all the six other piestine genera. This work found support for monophyly of the large genus *Piestus* and for relationships among the genera. Bortoluzzi *et al*. (2017) did find support for monophyly of the genus *Hypotelus*, which formed a sister-group of the Osoriinae (represented by only one terminal taxon in his work).

2. JUSTIFICATION

The problematic taxonomic history of Piestinae and some morphological uncertainties findings in previously published works (Caron *et al*. 2008, 2012; Grebennikov & Newton 2012; Bortoluzzi *et al*. 2017; Yamamoto *et al*. 2017), indicates the need for a targeted study with the goal of investigate the evolutionary history through the phylogenetic reconstruction, increasing the knowledge already available by Caron *et al*. (2012). Thus, as suggested by Grebennikov & Newton (2012), Piestinae should be re-evaluated and, probably, likely split into two or more monophyletic taxa of subfamily rank.

3. OBJECTIVE

Our original goals in this study were thus (1) to test the monophyly of Piestinae based on adult morphological data (Part I); (2) to test the monophyly of Piestinae based on larval morphological data (Part II); and (3) to test the monophyly of Piestinae based on molecular data (Part III). The choice to divide the goals in this way was based on the results presented by Grebennikov & Newton (2012) (non-monophyly of Subfamily Piestinae). Our main objective with this was to verify if, in any of the datasets, the Subfamily could be recovered as a monophyletic group.

As our work progressed, preliminary results indicated that Piestinae, as delimited in the terminals used in this work, is a monophyletic assemblage, although with some incongruences in their internal relation, mainly with respect to the genus *Siagonium*. We, therefore, widened the scope of our work with the goal (4) to test the monophyly of Piestinae based on merged morphological data in order to elucidate the internal relationships of the Subfamily (Part IV). This necessitated merging the two morphological data matrices.

4. PART I – MONOPHYLY OF PIESTINAE BASED ON ADULT MORPHOLOGICAL DATA

4.1. MATERIAL AND METHODS

4.1.1. Examined material

The material studied (Appendix 1) belongs to the following collections: Coleção Entomologica Pe. J. S. Moure, Universidade Federal do Paraná, Curitiba (DZUP, Lúcia Massutti de Almeida); Field Museum Natural History, Chicago (FMNH, Crystal Maier); Museu de Zoologia da Universidade de São Paulo, São Paulo, (MZUSP, Sônia Aparecida Casari).

We received the specimens previously identified by institutions, and their identifications were adopted as correct. In a few cases, when the material received was not identified (for example, some specimens of *Piestoneus*) or when the material was from field sampling (for example, some specimens of Aleocharinae, Oxytelinae, Osoriinae and Piestinae), the identifications were made by S. Bortoluzzi and E. Caron.

This study was conducted at the Laboratório de Pesquisa em Coleoptera (LAPCol), Departamento de Biodiversidade, Universidade Federal do Paraná–Setor Palotina, Palotina, Paraná, Brasil.

4.1.2. Choice of in-group and out-group taxa

The choices of all terminal taxa included in the phylogenetic analysis were based on the phylogenetic proposal of Grebennikov & Newton (2012). The relationship among the subfamilies showed by the authors (Scaphidiinae as sister-group of all remain subfamilies of the Oxyteline Group *sensu stricto*) was accepted by us as an a *priori* underlying hypothesis.

Representatives of all subfamilies of beetles currently assigned to the Oxyteline Group *sensu stricto* (Scaphidiinae, Oxytelinae, Osoriinae and Piestinae; see Grebennikov & Newton 2012) were included in the analysis (Table 2; Appendix 1; Fig.

3). According to Nixon & Carpinter (1993), we also included representatives of Staphylininae and Aleocharinae as out-group to test the polarization of character transformations in the analyses, once the phylogenetics systematics position of the subfamily Piestinae in relation to other subfamilies, which composes the Oxyteline Group, is doubtful.

The out-group sampling comprises species of the subfamilies belonging to the Oxyteline Group and of Staphylininae and Aleocharinae. Whenever possible the taxa sampling included at least one representative of each tribe of each subfamily; All tribes of Osoriinae was sampled; as well as three of the five tribes of Oxytelinae; and two of three tribes of Scaphidiinae. The ingroup species sampling comprises six genus-level taxa representatives of the seven extant genera. The genus *Parasiagonum* (monotypic) was not included in this study because of the limited number of specimens housed in the museum and, also, the impossibility of dissecting the material.

All species taxa utilized in this work were selected according to the availability of the material deposited in the institutions mentioned earlier or because of the ease of collecting them in the field.

4.1.3. Morphological terminology

The terminology and morphological interpretations herein adopted were based on Naomi (1987–1990; 2014) and Grebennikov & Newton (2012).

The measurements were given as the maximum length. Some diagnostic characters are shown on the figures using arrows.

Several characters were adopted from Grebennikov & Newton (2012) keeping their terminology. However, when there were conflicts between the used nomenclatures, in most of the cases the terminology proposed by Naomi was put on precedence.

Table 2. List of 36 adult terminals taxa included in the phylogenetic analysis to detect the monophyly of the subfamily Piestinae.

Family	Group	Subfamily	Tribe	Species
Out-group (18spp.):				
Staphylinidae	Staphylinine	Staphylininae	n/a	*Philonthus* sp.
Staphylinidae	Tachyporine	Aleocharinae	n/a	*Aleochara bonariensis*
Staphylinidae	Oxyteline	Scaphidiinae	Scaphisomatini	*Scaphisoma* sp.
Staphylinidae	Oxyteline	Scaphidiinae	Scaphidiini	*Scaphium castanipes*
Staphylinidae	Oxyteline	Oxytelinae	Deleasterini	*Oxypius peckorum*
Staphylinidae	Oxyteline	Oxytelinae	Bledini	*Bledius hermani*
Staphylinidae	Oxyteline	Oxytelinae	Oxytelini	*Ochthephilus biimpressus*
Staphylinidae	Oxyteline	Oxytelinae	Oxytelini	*Carpelimus* sp.
Staphylinidae	Oxyteline	Oxytelinae	Oxytelini	*Oxytelus* sp.
Staphylinidae	Oxyteline	Osoriinae	Eleusini	*Eleusis humilis*
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Allotrochus marginatus*
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Lispinus* sp.
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Nacaeus* sp.
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Thoracophorus sculptus*
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Glyptoma* sp.
Staphylinidae	Oxyteline	Osoriinae	Leptochirini	*Leptochirus scoriaceus*
Staphylinidae	Oxyteline	Osoriinae	Osoriini	*Osorius* sp.
Staphylinidae	Oxyteline	Osoriinae	Osoriini	*Holotrochus newtoni*
In-group (18 spp.):				
Staphylinidae	Oxyteline	Piestinae	n/a	*Eupiestus feae*
Staphylinidae	Oxyteline	Piestinae	n/a	*Eupiestus scupticolis*
Staphylinidae	Oxyteline	Piestinae	n/a	*Hypotelus castaneus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Hypotelus pusillus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestoneus lewisii*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestoneus monticola*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestoneus oharai*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestus bicornis*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestus mexicanus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestus minutus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestus sulcatus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Prognathoides mjobergi*
Staphylinidae	Oxyteline	Piestinae	n/a	*Siagonium debile*
Staphylinidae	Oxyteline	Piestinae	n/a	*Siagonium haroldi*
Staphylinidae	Oxyteline	Piestinae	n/a	*Siagonium nobile*
Staphylinidae	Oxyteline	Piestinae	n/a	*Siagonium punctatum*
Staphylinidae	Oxyteline	Piestinae	n/a	*Siagonium quadricorne*
Staphylinidae	Oxyteline	Piestinae	n/a	*Siagonium vittatum*

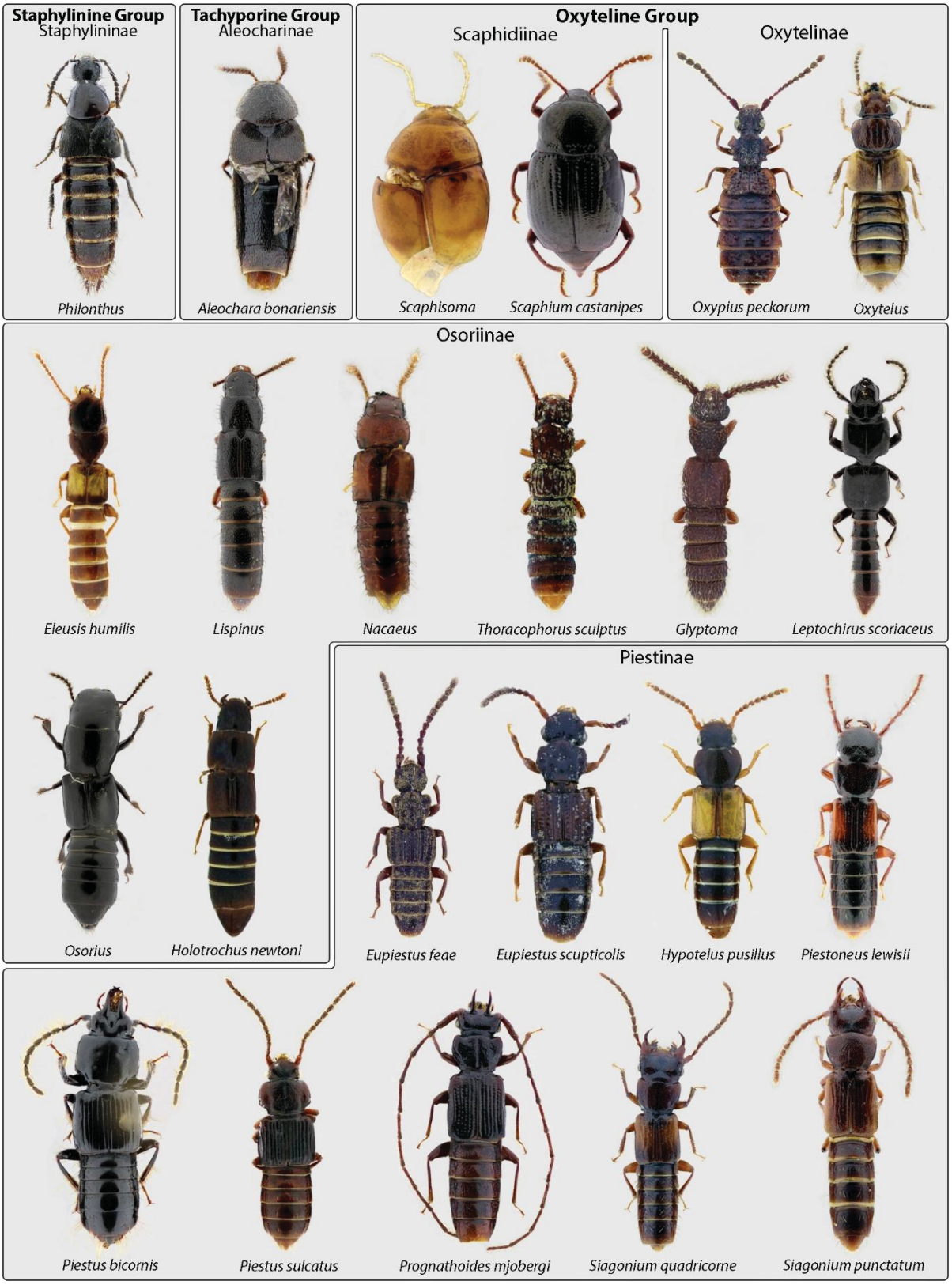

Figure 3. Adult habitus of some terminal taxa included in the phylogenetic analysis.

4.1.4. Preparation and illustration

The adult specimens were first macerated in a double boiler for 2–3 minutes for cleaning. The whole specimen was subsequently cleared in hot 10% KOH solution for 1–2 minutes (or more) according to the body size, then washed in glacial acetic acid and dissected in glycerol on Kline concavity slides.

Some adult specimens were partly disarticulated, allowing for free manipulation and rotation of the body parts, and stored in glycerol on temporally microscope slides. After the morphological study, the dissected parts were fixed in Canada balsam on acetate plastic cards.

Habitus images were taken using a Canon EOS 80D camera in conjunction with a Canon MP-E 65mm f/2.8 1-5X Macro Lens. Images of the dissected parts were taken using a Canon EOS 80D in conjunction with a 10X Infinity Plan Achromatic Microscope Objective mounted on a Canon EF 28-135mm f/3.5-5.6 IS USM Lens. Image Fig.4D was taken using a Moticam 5 CMOS digital camera attached on a Nikon Eclipse E200 light microscope. Images Figs. 4G, 5A were taken using a Moticam 5 CMOS digital camera attached to a Leica EZ4. Scanning electron microscope imaging was taken with a Tescan Vega 3 at Laboratório de Microscopia Eletrônica de Varredura, UFPR.

Image stacks were processed using Helicon Focus version 6.7.1. Final editing was performed using Adobe Photoshop CC software, version 2015.0.0.

4.1.5. Phylogenetic analyses

The matrix was edited in the program NEXUS Data Editor, version 0.5.0 (Page 2001) and comprise 36 terminal taxa, 18 from in-group and 18 from out-group. Characters not observed were coded as ‘?’ and those not applicable were coded as ‘-’.

The parsimony analyses were carried out using the program TNT version 1.5-beta (Goloboff *et al*. 2008a), with all characters being treated as unordered following the Fitch parsimony (Fitch 1971) as the criterion of optimization. All characters were non-additive and unordered. The genus *Philonthus* Stephens, 1829 (Staphylininae) was used to root the trees.

The parameters utilized in all searches were as follows: ‘max. tree’=500 000; ‘random seed’=1; ‘number of additional sequences’=50 000; ‘tree to save per replication’=10, utilizing ‘tree bisection reconnection’ (TBR) as the permutation algorithm of the branches. After obtaining the trees, a new TBR analysis was performed from the suboptimal trees ‘tree from RAM.’ The consistency (CI) and retention (RI) indices were recovered to compare the trees obtained.

In subsequent analyses, heuristic searches using implied weights (IW) (Goloboff 1993; Goloboff *et al*. 2008b) were conducted with the same parameters as above.

TNT scripts aaa.run and aab.run, written by Mirande (2009), were used to calculate the “best” value for the constant K. The values of K used in the analysis under IW were those that assign to an ‘‘average’’ character fits from 50 to 90% of the fit of a perfectly hierarchic one, producing 15 groups of trees obtained at each *K*-value (from 0 to 14, see Appendix 2). The best topology was chosen with the comparison between results using Farris distortion coefficient (Farris 1989), Robinson-Foulds distance (Robinson & Foulds 1981) and distance SPR (Goloboff 2008).

Branch support was rated using Bremer support (BS) (Bremer, 1994) and Bootstrap (BT) (Felsenstein 1985) with 10,000 replicates. Absolute Bremer support (ABS) was utilized for equal weighting analysis using the settings as in Leivas *et al*. (2014): traditional search (as explained earlier)> memory (500 001)>suboptimal (one step longer than the most-parsimonious trees)>traditional search (trees form RAM, stop maxtrees when hit)>Bremer support (absolute support)>check and note which clades were lost>repeat this procedure, gradually increasing the memory and suboptimal values, until all clades of interest were lost. ‘Retain trees suboptimal by’=16 steps were used. For the IW analysis, relative BS (RBS) was used (Goloboff & Farris 2001) with the following criterion: ‘Retain trees suboptimal by’=16 steps; ‘Relative fit difference’=1.00, as well as Bootstrap with 10,000 replicates.

Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The Mkv+Gmodel (Lewis 2001) was employed for the analysis, and two Markov chain Monte Carlo (MCMC) runs of four chains were run for ten million generations.

The convergence was determined to have occurred when the standard deviation of split frequencies dropped below 0.005, also by the effective sample size (ESS) values higher than 200 in Tracer v.16 (Rambaut *et al*. 2014), as well as by the

examination of potential scale reduction factor (PSRF) values in the MrBayes output file.

The first 25% of trees were discarded as burn-in. The 50% consensus tree of the two MCMC runs was rooted with *Philonthus*. Branch support was rated by posterior probability (PP). Branches with PP>0.95 were considered strongly supported; with PP=0.90–0.94 moderately supported; and with PP=0.85–0.89 weakly supported, as in Żyła *et al*. (2017).

The recovered topologies by TNT were manipulated and edited in WinClada version 1.00.08 (Nixon 2002). Trees from the Bayesian analysis was visualized and edited in Figtree v1.4.2 (Rambaut 2014). Final editing was performed using Adobe Illustrator CS6 version 16.0.0. Only unambiguous character changes are shown on the trees.

4.2. RESULTS

4.2.1. Character list

A total of 164 characters were examined and coded, including exoskeleton and endoskeleton. Characters of the aedeagus and spermatheca were not used. The following characters were scored as follows (Appendix 3):

Head:

0. *Head, posterior part of the head capsule, lateral constriction [dorsal view]*: (0) absent; (1) present. CI= 0.12, RI= 0.50.
1. *Head, posterior margin, occiput [dorsal view]*: (0) with a slightly or not projection at the mid; (1) with a conspicuous projection at the mid (Fig. 4A). CI= 0.14, RI= 0.64
2. *Head, dorsal transverse nuchal impression [dorsal view]*: (0) absent; (1) present (Fig. 4A). CI= 0.10, RI= 0.25. Character from Grebennikov & Newton (2012).
3. *Head, surface, mesial transversal sulcus [ventral view]*: (0) absent; (1) present. CI= 0.16, RI= 0.50.

4. *Head, dorsal tentorium arm, impression [dorsal view through clarified exoskeleton of the head]*: (0) dot, circle shape; (1) reniform shape. CI= 0.16, RI= 0.54. The character was examined with a microscope using transmitted light.
5. *Head, endoskeleton [ventral view through clarified exoskeleton of the head]*: (0) apparently not reaching the submentum; (1) reaching the submentum. CI= 0.25, RI= 0.62. The character was examined with a microscope using transmitted light.
6. *Head, frontoclypeal (=epistomal) suture [dorsal view]*: (0) absent; (1) present. CI= 0.33, RI= 0.60. Character from Grebennikov & Newton (2012).
7. *Head, eyes, insertion in the head, posterior face [dorsal view]*: (0) following the same line as the margin of the head, straight margin; (1) with cuticular projection of the head over the eye, inflexible margin (Fig. 4A). CI= 0.25, RI= 0.72.
8. *Head, ommatidia structure [dorsal view]*: (0) facets hexagonal and flat, eye surface smooth; (1) facets round and strongly convex, eye surface botryoidal. CI= 0.25, RI= 0.50. Character from Grebennikov & Newton (2012).
9. *Head, antennal insertion [dorsal view]*: (0) concealed under a ridge or a shelf-like elevation at sides of frons; (1) fully or partly exposed. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
10. *Antenna [dorsal view]*: (0) not geniculate; (1) geniculate (abruptly bent > 90°). CI= 0.50, RI= 0. Character from Grebennikov & Newton (2012).
11. *Antenna, antennomere 1 length ratio to antennomeres 2–3 combined*: (0) evidently shorter; (1) slightly equal; (2) evidently longer. CI= 0.20, RI= 0.55.
12. *Antenna, antennomere 3 length ratio to antennomere 2*: (0) evidently shorter; (1) slightly equal; (2) evidently longer. CI= 0.18, RI= 0.47.
13. *Antenna, antennomere 4 length ratio to antennomere 5*: (0) evidently shorter; (1) slightly equal; (2) evidently longer. CI= 0.25, RI= 0.62.
14. *Antenna, setigerous protuberances on antennomeres, even if reduced*: (0) absent; (1) present. CI= 0.25, RI= 0.76.
15. *Antenna*, *macrosetae, most of segments*: (0) only on apical half of antennomeres; (1) on over all antennomeres. CI= 0.11, RI= 0.38.
16. *Labrum [dorsal view]*: (0) not bilobed; (1) bilobed. CI= 0.25, RI= 0.62. Character from Grebennikov & Newton (2012).
17. *Mandibles*, *mandibular apices in apposition [dorsal view]*: (0) concealed beneath labrum; (1) fully exposed. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).

18. *Mandible*, *glandular cavities*: (0) absent; (1) present. CI= 0.50, RI= 0.50. Character from Grebennikov & Newton (2012).
19. *Mandible*, *mandibular molar lobe [dorsal view]*: (0) smooth, molar lobe not so projected; (1) well-development (Fig. 6D). CI= 0.14, RI= 0.40.
20. *Mandible, complete transversal sulcus through molar region [dorsal view]*: (0) absent; (1) present (Fig. 6D). CI= 1, RI= 1.
21. *Mandible, shape of the apical third of the prostheca line*: (0) straight; (1) curved; (2) parabola; (3) sinuous. CI= 0.27, RI= 0.52.
22. *Maxilla, palpifer, dorsal projection behind of the base of palpomeres [ventral view]*: (0) absent; (1) present. CI= 0.33, RI= 0.60.
23. *Maxilla, maxillary palpomere*: (0) 4 the longest; (1) 2 the longest; (2) 3 the longest. CI= 0.40, RI= 0.
24. *Maxilla, maxillary palpomere 4, impressions near apex*: (0) absent; (1) present (FIG. 6H). CI= 0.33, RI= 0.80.
25. *Maxilla, general aspect of the maxillary palpomeres*: (0) glabrous, few seated; (1) setaceous. CI= 0.33, RI= 0.33.
26. *Maxilla, lacinia, apical unarticulated spine [ventral view]*: (0) absent; (1) present. CI= 0.16, RI= 0.16. Character from Grebennikov & Newton (2012).
27. *Maxilla, lacinia, setae*: (0) setiform; (1) spiniform. CI= 0.20, RI= 0.63.
28. *Maxilla, lacinia, setae, aspect:* (0) plumose; (1) simple. CI= 0.50, RI= 0.83.
29. *Maxilla, lacinia, outer margin where the setae are inserted*: (0) hard sclerotized; (1) soft sclerotized, looks membranous (Fig. 4C). CI= 0.33, RI= 0.33.
30. *Labium, palpomere 1, seta near of the apical margin*: (0) very short, inconspicuous, shorter than palpomere 2; (1) long, evidently, as long as to longer than palpomere 2. CI= 0.16, RI= 0.37.
31. *Labium, palpomere 1, small pit near the seta of the apical margin*: (0) absent; (1) present. CI= 0.20, RI= 0.76.
32. *Labium, palpomere 2*: (0) longer than wide; (1) wider than long. CI= 0.14, RI= 0.40.
33. *Labium, palpomere 3, length*: (0) not longer than 1 and 2 combined; (1) longer than 1 and 2 combined. CI= 0.12, RI= 0.12.
34. *Ligula, median sclerite*: (0) absent; (1) present (Fig. 4D). CI= 0.25, RI= 0.25.
35. *Ligula, median sclerite*: (0) not reaching the anterior margin of the ligula; (1) reaching the anterior margin of the ligula (Fig. 4D). CI= 0.20, RI= 0.63.

36. *Ligula, median sclerite, apical region*: (0) acute (Fig. 4D); (1) broad. CI= 0.16, RI= 0.50.
37. *Ligula, macrosetae (one or two pairs) near of the median sclerite*: (0) absent; (1) present (Fig. 4D). CI= 0.20, RI= 0.20.
38. *Ligula*, *macrosetae near of the median sclerite, shape*: (0) acuminate (Fig. 4D); (1) enlarged. CI= 0.25, RI= 0.40.
39. *Ligula, two sclerites by each side of the median sclerite*: (0) absent; (1) present (Fig. 4D). CI= 0.33, RI= 0.80.
40. *Mentum [ventral view]*: (0) subquadrate or elongate, < 1.5 × as wide as long; (1) transverse, ≥ 1.5 × as wide as long. CI= 0.33, RI= 0.75. Character modified from Grebennikov & Newton (2012).
41. *Mentum, deep impressed at least near of its antero-laterad margins [ventral view]*: (0) absent; (1) present. CI= 1, RI= 1. Note: *Oxytelus* sp. has one semicircular impression on basal half of the mentum, but it is not at the same position as described in the character 41. So, it was considered as absent for *Oxyletus* sp.
42. *Mentum, antero lateral margin, number of long setae*: (0) nil; (1) one; (2) two; (3) three; (4) more than three. CI= 0.28, RI= 0.47.
43. *Submentum [ventral view]*: (0) small; (1) large (Fig. 4B). CI= 0.50, RI= 0.50.
44. *Submentum and gula [ventral view]*: (0) separated by internal ridge anterior to posterior tentorial pits; (1) not separated by internal ridge. CI= 0.16, RI= 0.37. Character from Grebennikov & Newton (2012).
45. *Gular sutures [ventral view]*: (0) separate throughout (Fig. 4B); (1) fused for part of their length. CI= 0.16, RI= 0.61.
46. *Gular sutures (or suture, if single) [ventral view]*: (0) incomplete, obsolete in anterior 1/3 or 1/2; (1) complete, extending anteriorly to buccal cavity (Fig. 4B). CI= 0.50, RI= 0.67. Character modified from Grebennikov & Newton (2012).
47. *Gular plate, deep depression at the middle [ventral view]*: (0) absent; (1) present. CI= 0.33, RI= 0.80.
48. *Deep impression on surface of head at connection of the submentum with the end of gular plate [ventral view]*: (0) absent; (1) present (Fig. 4B). CI= 1, RI= 1.

Thorax:

49. *Prothorax, cervical sclerites, apex format*: (0) not bilobed; (1) bilobed (Fig. 4A). CI= 0.50, RI= 0.92.

50. *Pronotum, anterior margin, width in relation to posterior margin [dorsal view]*: (0) narrower; (1) subequal; (2) wider (Fig. 4F). CI= 0.28, RI= 0.28.
51. *Prothorax, pronotum, front angles, relative to the anterior margin of pronotum [dorsal view]*: (0) not produced anterad (Fig. 4F); (1) produced anterad (Fig. 4E). CI= 0.16, RI= 0.66.
52. *Prothorax, pronotum, internal mid-longitudinal ridge or projection [dorsal view]*: (0) absent; (1) present, from single projection to short ridge less than half pronotal length; (2) present, half of pronotal length or longer (Fig. 4F). CI= 0.33, RI= 0.63. Character from Grebennikov & Newton (2012).
53. *Prothorax, pronotum, pronotosternal suture [ventral view]*: (0) absent or very incomplete and evident only posteriorly near coxal cavity; (1) present, ± complete as fine groove or carina, not membranous; (2) present, complete, distinctly membranous (Fig. 4E). CI= 0.18, RI= 0.52. Character from Grebennikov & Newton (2012).
54. *Prothorax, pronotum, furcasternum, distance between the two mesially endoskeletons*: (0) shorter than length of the endoskeleton (Fig. 4H); (1) longer than length of the endoskeleton. CI= 0.12, RI= 0.50.
55. *Prothorax, pronotum, second projection of the furcasternum, behind of furcasternum+prosternal process, even slightly*: (0) absent; (1) present (Fig. 4G). CI= 0.50, RI= 0.92.
56. *Prothorax, pronotum, prosternal+furcasternal process, apex length:* (0) not projected beyond apex of hypomeral projection; (1) projected beyond apex of hypomeral projection. CI= 0.16, RI= 0.37.
57. *Prothorax, prosternum, internal oval posterior impression, from insertion of furcasternum process [ventral view through clarified exoskeleton of the head]:* (0) absent; (1) present (Fig. 4E). CI= 0.33, RI= 0.86.
58. *Prothorax, prosternum, internal oval anterior impression, from insertion of cervical sclerites process [ventral view through clarified exoskeleton of the pronotum]:* (0) absent; (1) present (Fig. 4E). CI= 0.14, RI= 0.45.
59. *Prothorax, prosternum, internal anterior transversal carina [view through anterior foramen]*: (0) slight prominent; (1) large, evidently prominent (Fig. 4H). CI= 0.25, RI= 0.70.

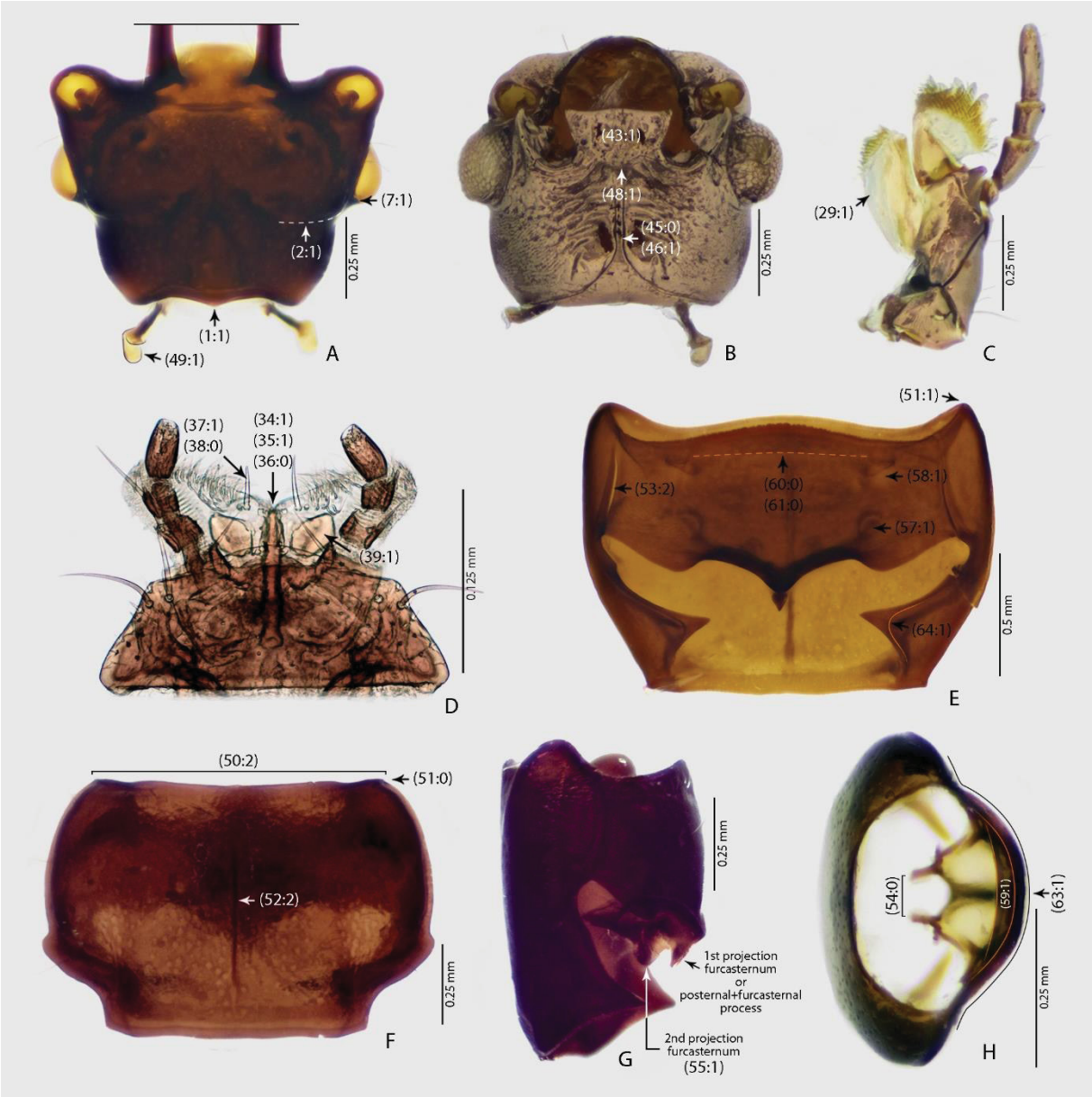

Figure 4. Adult morphological characters of some terminal taxa included in the phylogenetic analyses: head and prothorax. (A) *Prognathoides mjobergi*, male; (B) *Piestus sulcatus*, male; (C, E, G) *Piestoneus lewisii*, male; (D) *Siagonium punctatum*, male; (F) *P. sulcatus*, male; (H) *Hypotelus pusillus*, male. (A) head, dorsal; (B) head, ventral; (C) maxilla, ventral; (D) labium, ventral; (E) pronotum, ventral; (F) pronotum, dorsal; (G) pronotum, lateral; (H) pronotum anterior.

60. *Prothorax*, *internal anterior transversal carina, shape [ventral view through clarified exoskeleton of the pronotum]*: (0) line transversal (Fig. 4E); (1) line sinuous. CI= 0.50, RI= 0.83.

61. *Prothorax, internal transversal carina [ventral view through clarified exoskeleton of the pronotum]*: (0) follow anterior margin of prosternum (Fig. 4E); (1) directed to the middle of prosternum. CI= 0.14, RI= 0.60.
62. *Prothorax*, *anterior margin of prosternum, shape at the middle*: (0) straight; (1) projected. CI= 0.20, RI= 0.69.
63. *Prothorax, prosternum [anterior view]*: (0) middle not prominent in relation to sides; (1) middle prominent in relation to sides (Fig. 4H). CI= 0.24, RI= 0.66.
64. *Prothorax, hypomeral projection, marginal carina*: (0) absent; (1) present (Fig. 4E). CI= 0.16, RI= 0.44.
65. *Anterior leg, protrochantin [lateral view]*: (0) concealed; (1) exposed. CI= 0.20, RI= 0.55. Character from Grebennikov & Newton (2012).
66. *Anterior leg, external margins of procoxae [ventral view]*: (0) contiguous or subcontiguous; (1) well separated by prosternal process, even if internally subcontiguous. CI= 0.20, RI= 0.20. Character from Grebennikov & Newton (2012).
67. *Elytra, elytral lenght*: (0) long, covering most of tergal abdominal segments; (1) short, exposing most of tergal abdominal segments. CI= 1, RI= 1.
68. *Elytra, elytral striation, sutural stria [dorsal view]*: (0) absent; (1) present. CI= 0.20, RI= 0.33. Character from Grebennikov & Newton (2012).
69. *Elytra, elytral striation, sutural stria [dorsal view]*: (0) punctate; (1) striate; (2) punctate and striate. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
70. *Elytra, elytral striation, length of sutural stria*: (0) not reaching marginal apex of elytra; (1) reaching or close to marginal apex of elytra. CI= 0.33, RI= 0.84.
71. *Elytra, elytral striation, ending of sutural stria, aspect*: (0) straight; (1) curved. CI= 0.33, RI= 0.50.
72. *Elytra, elytral striation, non-sutural striae, aspect [dorsal view]*: (0) absent; (1) present. CI= 0.20, RI= 0.75. Character from Grebennikov & Newton (2012).
73. *Elytra, elytral striation, non-sutural striae [dorsal view]*: (0) punctate; (1) striate; (2) punctate and striate (Fig. 6F). CI= 0.40, RI= 0.57. Character from Grebennikov & Newton (2012).
74. *Elytra, elytral epipleural ridge [lateral view]*: (0) absent; (1) present. CI= 0.33, RI= 0.33. Character from Grebennikov & Newton (2012).
75. *Elytra, carina on marginal apex, fused with sutural stria*: (0) absent; (1) present. CI= 0.16, RI= 0.28

76. *Elytra, posterior margin, keel [dorsal view]*: (0) absent; (1) present (Fig. 6F). CI= 1, RI= 1.
77. *Elytra, apical inner margin, small projection of elytral suture*: (0) absent; (1) present (Fig. 6F). CI= 0.50, RI= 0.90.
78. *Elytra, basolateral angle, small acute tooth [dorsal view]*: (0) absent; (1) present (Fig. 6F–G). CI= 0.50, RI= 0.75. Character from Caron *et al*. (2012).
79. *Elytra, humeral angle [dorsal view]*: (0) rounded (Fig. 6F–G); (1) quadrate. CI= 0.33, RI= 0.50.
80. *Elytra, internal elytral spine, base [ventral view]*: (0) fused to the elytrum, following the direction of the margin, curved (Fig. 6G); (1) fused to the elytrum, not following the direction of the margin, straight. CI= 0.25, RI= 0.57.
81. *Elytra, internal elytral spine, apex [ventral view]*: (0) not distinct, gradually fused to the elytrum; (1) distinct, not fused to the elytrum (Fig. 6G). CI= 0.25, RI=0.70.
82. *Elytra, impression at the base of the spine of the elytrum*: (0) absent; (1) present. CI= 0.14, RI= 0.57.
83. *Wing, veins pattern [dorsal view]*: (0) weak sclerotized, not so evident, more or less transparent; (1) strong sclerotized, well-visible (Fig. 5A). CI= 0.20, RI= 0.55.
84. *Wing, anal lobe (= region after vein A1+A2) [dorsal view]*: (0) not so developed; (1) well-developed (Fig. 5A). CI= 0.20, RI= 0.50.
85. *Wing, anal lobe, angle [dorsal view]*: (0) not strongly angulated; (1) strongly angulated, almost 90º (Fig. 5A). CI= 0.16, RI= 0.44.
86. *Wing, anal lobe, additional lobule (=alula, Diptera) [dorsal view]*: (0) absent; (1) present (Fig. 5A). CI= 0.25, RI= 0.40.
87. *Wing, vein A1+A2*: (0) not divided; (1) divided. CI= 0.25, RI= 0.62.
88. *Wing, vein A1+A2*: (0) division occurs on anterior half, closer to base of the veins; (1) division occurs on apical third (Fig. 5A). CI= 1, RI= 1.
89. *Wing, vein Cu*: (0) apex closer to vein A1; (1) apex closer to apex of the wing, in a straight line (Fig. 5A). CI= 0.11, RI= 0.20.
90. *Mesothorax, transverse anepisternal (=mesepisternal) carina, even if incomplete [ventral view]*: (0) absent; (1) present. CI= 0.12, RI= 0.22. Character from Grebennikov & Newton (2012).
91. *Mesothorax, carina delimiting prepectus from anepisternum, even if incomplete [ventral view]*: (0) absent; (1) present. CI= 0.33, RI= 0.33. Character from Grebennikov & Newton (2012).

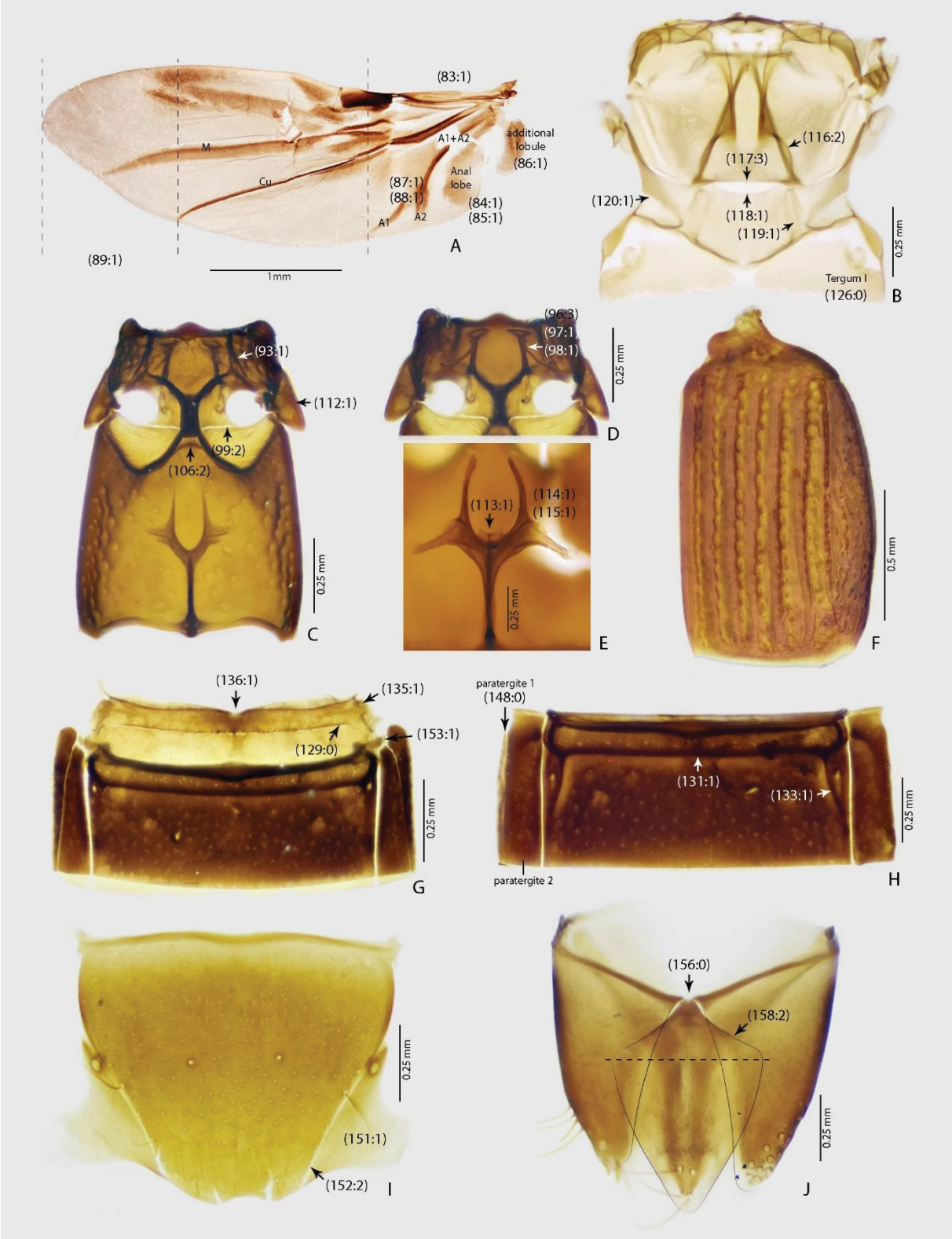

Figure 5. Adult morphological characters of some terminal taxa included in the phylogenetic analyses: pterothorax and abdomen. (A, E, J) *Piestus bicornis*, female; (B–D) *Prognathoides mjobergi*, male; (F) *Piestus mexicanus*, male; (G–I) *Piestoneus lewisii*, male. (A) wing, lateral; (B) pterothorax + abdominal tergum I, dorsal; (C) meso-

metaventrite, ventral; (D) mesoventrite, internal; (E) metendoesternite; (F) elytrum, dorso-lateral; (G, H) terga II–IV, dorsal; (I) tergum VIII, dorsal; (J) terga IX–X, dorsal.

92. *Mesothorax, whether modified for external closure of procoxal cavities [ventral view]*: (0) not modified; (1) modified, with anterior excavation for each procoxa. CI= 1, RI= 1.
93. *Mesothorax, anapleural suture, even if incomplete [ventral view]*: (0) absent; (1) present. CI= 0.16, RI= 0.68. Character from Grebennikov & Newton (2012).
94. *Mid-leg, mesotrochantin [lateral view]*: (0) concealed; (1) exposed (Fig. 5C). CI= 0, 25, RI= 0.66. Character from Grebennikov & Newton (2012).
95. *Mid-leg, mesocoxae [ventral view]*: (0) contiguous; (1) narrowly separated by third or less coxal width; (2) widely separated by at least half of coxal width. CI= 0.33, RI= 0.42. Character from Grebennikov & Newton (2012).
96. *Mesothorax, mesendosternites, shape*: (0) one pair of vertical stick; (1) one pair of vertical stick curved anteriorly, forming 90° angle; (2) one pair of vertical stick curved at apex and enlarged at base; (3) one pair of vertical stick and one projection curved posterad (Fig. 5D). CI= 0.75, RI= 0.83.
97. *Mesothorax, mesendosternites, projection curved posterad (state 3 from previous character), attachment:* (0) at apex, anteriorly; (1) anterior half, near the apex (Fig. 5D); (2) at half posterior; (3) at the middle. CI= 0.42, RI= 0.60.
98. *Mesothorax, mesendosternites, muscle disk [ventral view through translucent mesoventrite]*: (0) absent; (1) present (Fig. 5D). CI= 0.25, RI= 0.66. Character from Grebennikov & Newton (2012).
99. *Mesothorax, meso-metaventral suture [ventral view]*: (0) fused by their length; (1) delimited by ridge; (2) separated by fine membrane (Fig. 5C). CI= 0.20, RI= 0.27. Character from Grebennikov & Newton (2012).
100. *Mesothorax, mesoventrite process in relation to metaventrite process*: (0) shorter in length; (1) equal in length; (2) longer in length. CI= 0.28, RI= 0.37.
101. *Mesothorax, mesoventrite process*: (0) not projected beyond the apex of metaventrite process; (1) projected beyond the apex of metaventrite process. CI= 0.12, RI= 0.50.
102. *Mesothorax, mesoventrite process, apex*: (0) truncate; (1) rounded; (2) pointed. CI= 0.15, RI= 0.35.

103. *Mesothorax, mesoventreite process, evident median carina*: (0) absent; (1) present. CI= 0.50, RI= 0.83. Character from Caron *et al*. (2012).
104. *Metathorax, metaventrite process in relation to mesoventrite process, apex*: (0) not wider; (1) wider. CI= 0.20, RI= 0.66.
105. *Metathorax, mid coxal cavity, carina*: (0) absent; (1) present. CI= 1, RI= 1.
106. *Metathorax, metaventrite, transverse carina behind mesocoxae [ventral view]*: (0) absent; (1) present, interrupted at middle; (2) present, complete (Fig. 5C). CI= 0.40, RI= 0. Character from Grebennikov & Newton (2012).
107. *Metathorax, metaventrite, sides, longitudinal carina*: (0) absent; (1) present. CI= 0.33, RI= 0.
108. *Metathorax*, *mid coxal cavity, transversal sclerite [internal view]*: (0) absent; (1) present. CI= 0.16, RI= 0.66.
109. *Metathorax*, *mid coxal cavity, transversal sclerite (if previous character present), shape [internal view]*: (0) transaversal ridge; (1) pointed projection; (2) carined projection. CI= 0.66, RI= 0.66.
110. *Metathorax, mid coxal cavity (at metasternite region) [dorsal view]*: (0) contiguous; (1) separated. CI= 0.20, RI= 0.42.
111. *Metathorax, metaventrive, mesal posterior lobes [ventral view]*: (0) absent; (1) present. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
112. *Metathotax, Epimeron 2, longitudinal ventral delimitation*: (0) slight folding; (1) evident folding (Fig. 5C). CI= 0.20, RI= 0.69.
113. *Metathorax, metendoesternite, median process*: (0) absent; (1) present (Fig. 5E). CI= 0.25, RI= 0.70.
114. *Metathorax, metendoesternite, furcal arms*: (0) not divided; (1) divided in two branches (Fig. 5E). CI= 0.25, RI= 062.
115. *Metathorax, metendoesternite, if furcal arms divided*: (0) anterior branch shorter than posterior; (1) anterior branch longer than posterior (Fig. 5E). CI= 0.25, RI= 0.25.
116. *Pterothorax, alacrista (Naomi 1988: fig. 3A; Morphology part VI) [dorsal view]*: (0) straight vertically; (1) straight oblique; (2) curved concave (Fig. 5B). CI= 0.66, RI= 0.75.
117. *Pterothorax, scutellum (Naomi 1988: fig. 3B; Morphology part VI), posterior margin [dorsal view]*: (0) straight; (1) emarginated; (2) projected; (3) straight and projected (Fig. 5B). CI= 0.25, RI= 0.43.

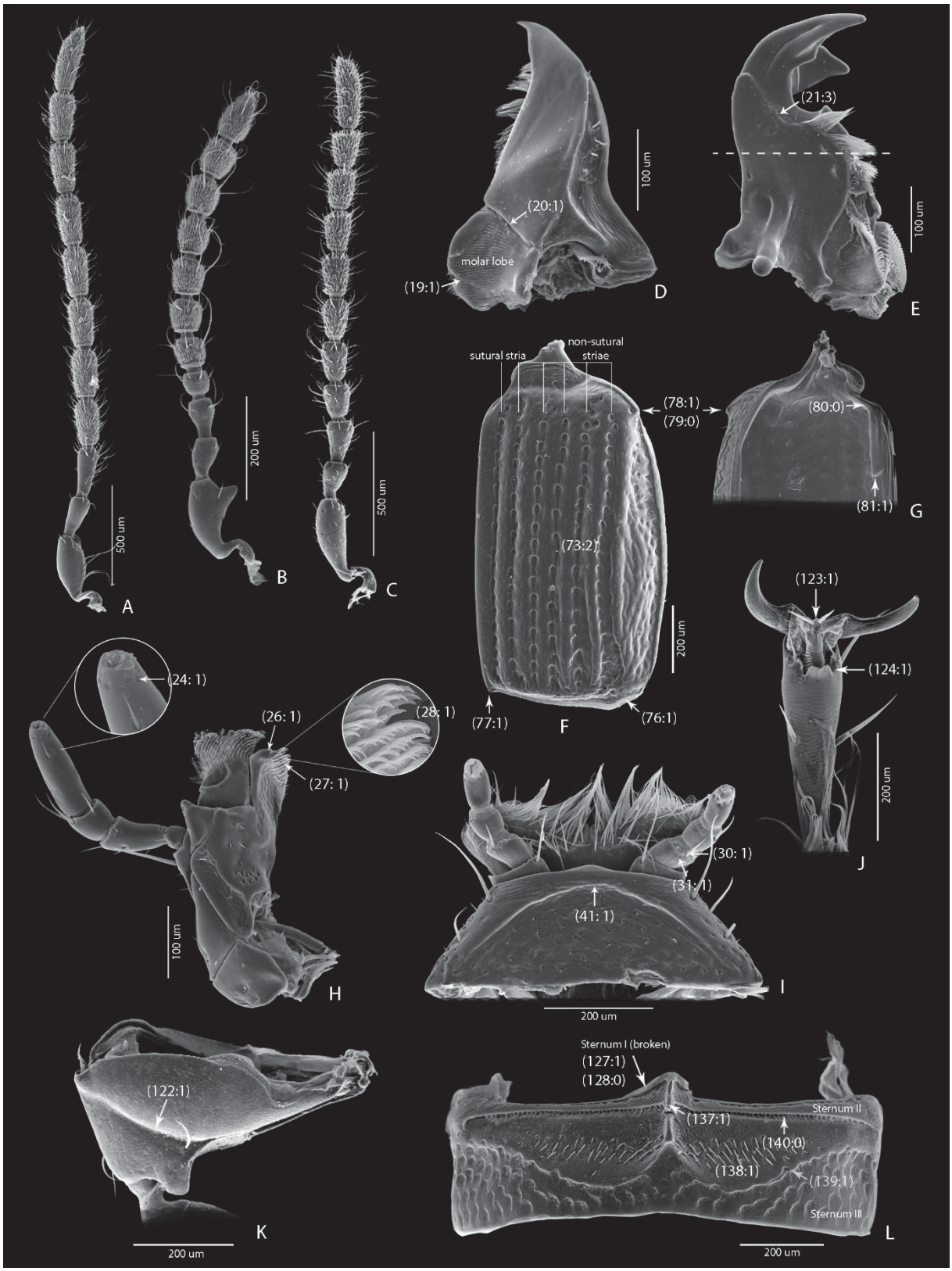

Figure 6. Adult morphological characters of some terminal taxa included in the phylogenetic analyses: Scanning Electron Microscopy. (A, F) *Piestus mexicanus*, male; (B) *Hypotelus pusillus*, male; (C, H) *Siagonium punctatum*, male; (D) *Oxypius peckorum*, male; (E) *Piestoneus oharai*, female; (G, K, L) *Piestus sulcatus*, male; (I)

Piestoneus lewisii, male; (J) *Piestus bicornis*, female. (A–C) antennae; (D) mandible, dorsal; (E) mandible, ventral; (F) elytrum, dorso-lateral; (G) elytrum, ventral; (H) maxilla, ventral; (I) labium, ventral; (J) apical tarsomere, ventral; (K) metacoxa, ventral; (L) sterna I–III, ventral.

118. *Pterothorax, postnotum (Naomi 1988: fig. 3B; Morphology part VI), anterior margin [dorsal view]*: (0) straight; (1) emarginated (Fig. 5B); (2) projected. CI= 0.18, RI= 0.47.
119. *Pterothorax, postnotum (Naomi 1988: fig. 3B; Morphology part VI), longitudinal suture [dorsal view]*: (0) absent; (1) present (Fig. 5B). CI= 0.20, RI= 0.42.
120. *Pterothorax, postnotum (Naomi 1988: fig. 3B; Morphology part VI), transversal suture [dorsal view]*: (0) by ridge; (1) by membrane (Fig. 5B). CI= 0.116, RI= 0.28.
121. *Anterior leg, procoxae, deep groove and carina*: (0) absent; (1) present. CI= 0.25, RI= 0.70.
122. *Hind leg, metacoxae, evident median transversal sulcus [ventral view]*: (0) absent; (1) present (Fig. 6K). CI= 0.14, RI= 0.60.
123. *Leg, empodial setae or setiform process*: (0) absent; (1) present (Fig. 6J). CI= 0.25, RI= 0.25.
124. *Leg, ventral process projecting over empodium, if present*: (0) not divided; (1) divided (Fig. 6J). CI= 0.16, RI= 0.68.
125. *Hind leg, metatarsus, length of last tarsomere [lateral view]*: (0) shorter than all previously combined tarsomeres; (1) subequal to all previously combined tarsomeres; (2) longer than all previously combined tarsomeres. CI= 0.33, RI= 0.60.

Abdomen:

126. *Tergum I [dorsal view]*: (0) divided from each other, forming a pair of triangular plates (Fig. 5B); (1) completely fused. CI= 0.12, RI= 0.50.
127. *Sternum I [dorsal view]*: (0) absent; (1) present. CI= 0.14, RI= 0.40.
128. *Sternum I, if present [ventral view]*: (0) fused in one plate; (1) divided in two plates. CI= 0.25, RI= 0.50.
129. *Tergum II, anterior transverse subbasal carina (=anterior transverse basal carina) [dorsal view]*: (0) absent or incomplete, not reaching the sides of the tergum (Fig. 5G); (1) complete, reaching sides of the tergum. CI= 0.14, RI= 0.60.

130. *Tergum III, anterior transverse subbasal carina (=anterior transverse basal carina) [dorsal view]*: (0) absent; (1) present. CI= 0.50, RI= 0.75. Character from Grebennikov & Newton (2012).
131. *Terga IV–VII, anterior transverse subbasal carina (=anterior transverse basal carina) [dorsal view]*: (0) absent; (1) presente (Fig. 5H). CI= 0.25, RI= 0.72. Character from Grebennikov & Newton (2012).
132. *Tergum III, basolateral ridges*: (0) absent; (1) present. CI= 0.33, RI= 0.80. Character from Grebennikov & Newton (2012).
133. *Basolateral ridges of abdominal terga IV-VII*: (0) absent; (1) present (Fig. 5H). CI= 0.25, RI= 0.72. Character from Grebennikov & Newton (2012).
134. *Sternum III, lateral longitudinal carina on each side:* (0) absent; (1) present. CI= 0.25, RI= 0.40. Character from Grebennikov & Newton (2012).
135. *Tergum II, spiracles, location [dorsal view]*: (0) in the membrane, at edges of tergum II; (1) in the tergum II (Fig. 5G). CI= 0.25, RI= 0.50.
136. *Tergum II, anterior margin at the middle, shape [dorsal view]*: (0) straight; (1) emarginated (Fig. 5G); (2) projected. CI= 0.25, RI= 0.60.
137. *Sterna II–III, intercoxal carina or elevation [ventral view]*: (0) absent; (1) present. CI= 0.33, RI= 0.71. Character from Grebennikov & Newton (2012).
138. *Sterna II–III, metacoxal excavations [ventral view]*: (0) absent; (1) present. CI= 0.25, RI= 0.62.
139. *Sterna II–III, metacoxal excavations, if present, transverse carina [ventral view]*: (0) absent; (1) present. CI= 0.33, RI= 0.81.
140. *Sterna II–III [ventral view]*: (0) solidly fused along most of their width; (1) separated by long connecting membrane permitting relative move. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
141. *Terga and sterna III-VII [lateral view]*: (0) not fused, separated by suture; (1) fused to form complete abdominal ring. CI= 0.50, RI= 0.85. Character from Grebennikov & Newton (2012).
142. *Abdominal segment II, paratergites [lateral view]*: (0) absent; (1) present. CI= 1, RI= 1.
143. *Abdominal segment II, paratergites, if present [lateral view]*: (0) one; (1) two. CI= 1, RI= 1.
144. *Abdominal segments III-VI, paratergites [lateral view]*: (0) absent; (1) present. CI= 0.50, RI= 0.87.

145. *Abdominal segments III-VI, paratergites, if present [lateral view]*: (0) one; (1) two. CI= 0.25, RI= 0.50.

146. *Abdominal segment VII, paratergites [lateral view]*: (0) absent; (1) present. CI= 0.50, RI= 0.88.

147. *Abdominal segment VII, paratergites, if present [lateral view]*: (0) one; (1) two. CI= 0.50, RI= 0.80.

148. *Abdominal segments III-VI, paratergites, if two presents*: (0) paratergite 1 slender than 2 (Fig. 5H); (1) paratergite 1 wider than 2. CI= 0.50, RI= 0. Note: number of the paratergites is in according to Naomi (2014).

149. *Tergum VII, laterotergites*: (0) absent; (1) present. CI= 0.50, RI= 0.85.

150. *Tergum VII, laterotergites, if present, demarcated from*: (0) indistinctly from tergum VII by fold; (1) distinctly from tergum VII by ridge. CI= 0.50, RI= 0.66.

151. *Tergum VIII, laterotergires*: (0) absent; (1) present (Fig. 5I). CI= 1, RI= 1.

152. *Tergum VIII, laterotergites, if present, demarcated from*: (0) indistinctly demarcated from tergum VIII by fold; (1) evidently demarcated from tergum VIII by fold; (2) distinctly demarcated from tergum VIII by suture (Fig. 5I); (3) partially demarcated by suture. CI= 0.42, RI= 0.77.

153. *Abdominal segment III, paratergite, small sclerite above paratergite*: (0) absent; (1) present (Fig. 5G). CI= 0.50, RI= 0.88.

154. *Abdominal apex, defensive (odoriferous) glands in the terminalia*: (0) absent; (1) present. CI= 1, RI= 1. Character modified from Grebennikov & Newton (2012).

155. *Sternum VII, posterolateral area, very small emargination (external openings of abdominal defensive gland complex)*: (0) absent; (1) present. CI= 1, RI= 1. Character from Caron *et al.* (2012).

156. *Tergum IX [dorsal view]*: (0) dorsally slightly divided by tergum X, distance between margins more or less gradually increasing posteriorly (Fig. 5J); (1) dorsally widely divided by tergum X; (2) dorsally contiguous, but margins widely separated. CI= 0.50, RI= 0.87. Character modified from Solodovnikov & Newton (2005).

157. *Tergum X, base, median longitudinal emargination (at least on female)*: (0) absent; (1) present. CI= 0.50, RI= 0.50.

158. *Tergum X, basal half, shape*: (0) subparallel sides; (1) ovoid; (2) triangulate, sides emarginated (Fig. 5J). CI= 0.66, RI= 0.94.

159. *Tergum IX male, ventral struts*: (0) absent; (1) present. CI= 0.25, RI= 0.57. Note: the characters of ventral struts listed here were considered as present or as absent comparing the male and female structure shape. If the shape is the same in both males and females, the state for the character was scored as absent. Note: this character was scored as "?" for *Lispinus* sp., because we had only male for this species.
160. *Tergum IX male, ventral struts, if present, shape*: (0) short, not so projected; (1) long and slender, strongly projected. CI= 1, RI= 1. Note: this character was scored as "?" for *Lispinus* sp., because we had only male for this species.
161. *Tergum IX male, ventral struts, if present, apex fused each other*: (0) absent; (1) present. CI= 1, RI= 1. Note: this character was scored as "?" for *Lispinus* sp., because we had only male for this species.
162. *Sternum IX male, base, shape*: (0) pointed; (1) enlarged. CI= 0.16, RI= 0.50. Note: this character was scored as "?" for *Osorius* sp. and *Allotrochus marginatus*, because we did not find the sternum IX.
163. *Abdominal segments III–VIII, posterior half enlarged for allocation of the adjacent abdominal segments*: (0) absent; (1) present. CI= 0.33, RI= 0.

4.2.2. Equal weighting versus implied weighting versus Bayesian analysis

Maximum parsimony (MP) analyses under equal weights (EW) returned four most parsimonious trees with a length of 729 steps, with CI= 0.27 and RI= 0.67. The topology of the strict consensus is shown in Fig. 8.

The analysis attributing IW generated only one tree for each *K* value utilized (see Appendix 2). According to the assumptions mentioned above, e. g., Material and Methods, and to the parameters obtained on the IW analyses (see Appendix 2), the best topology reached correspond to the one of *K5* (K value equal to 4.827). The analysis conducted with *K5* criterion generated one tree with 734 steps and 56.960 adjusted fit (Appendix 2), shown in Fig. 7. RBS was calculated from 13,752 suboptimal trees and is shown below each clade branch.

The Bayesian inference (BI) analysis (Fig. 9) converged before 10 million generations and, at the end of the run, an average standard deviation of split frequencies had stabilized well below 0.005, while nearly all PSRF values were 1.000

(minimum = 1.000; maximum= 1.002).

Although the EW analyses obtained a higher CI and RI values, and four parsimonious trees with less number of steps (729), the most parsimonious tree of the IW (*k5*) was the unique topology that recovered the four main lineages that compose the Oxyteline Group *sensu stricto*. Thus, below we discuss the common transformations of the IW analysis unless another state is indicated in the text. The differences between these two analyses are shown in the Tables 3–4. The main synapomorphies shared by the main clades are shown in the Table 4.

Among the four subfamilies composing the Oxyteline Group *sensu stricto*, four were found to be monophyletic on the reference tree (topology of analysis under IW with criterion *K5*; Fig. 7). On the results under EW, three subfamilies were recovered as a clade, while Osoriinae was found to be polyphyletic with respect to Scaphidiinae (Table 3; Fig. 8). In the BI, only Scaphidiinae and Piestinae were recovered as a monophyletic clade, both with strong support (PP= 1.00) (Table 3; Fig. 9).

About to the subfamily Piestinae, all analyses consistently recovered the group as a monophyletic clade, always with high support: EW with ABS= 13 and BT= 94; IW with RBS= 61 and BT= 98; and, BI with PP=1.00 (Table 3). The clade is supported by 16 common transformations, ten homoplastic [4:1; 7:1; 31:1; 32:1; 57:1; 70:0; 72:1; 89:1; 121:1; 162:0] and six synapomorphic [49:1; 69:2; 76:1; 156:0; 158:2; 160:0] (Table 4; Fig. 7).

The phylogenetic analyses recovered two main topologies to the Piestinae lineage. One topology shows the monotypic genus *Prognathoides* as sister-group to the all remain genera of Piestinae, e. g., topologies from IW analysis and some results from EW analysis (see Fig. 7; Appendix 4). Another topology shows the monotypic genus *Prognathoides* as sister-group to the lineage of *Siagonium* + *Piestoneus*, e. g., topologies from BI analysis and some results from EW analysis (see Fig. 9. Appendix 4).

Internal generic relationships within piestines rove beetles are variable, but the analyses consistently identified two main clades: *Piestus* + (*Hypotelus* + *Eupiestus*) and, *Siagonium* + *Piestoneus*. The last clade was weakly supported (ABS= 5; RBS= 40; BT= 68; PP= 0.60) (Table 3), sharing four common homoplastic transformations [24:1; 36:1; 47:1; 139:1] (Table 4; Fig. 7).

The first clade (*Piestus* + (*Hypotelus* + *Eupiestus*)) is supported by eight common transformations, seven homoplastic [54:1; 59:1; 61:1; 108:1; 113:1; 122:1;

129:1] and one synapomorphic [88:1] (Table 3; Fig. 7). However, this clade has not been well supported in all analyses by the all criterions of support (Table 2). By the contrast, the clade of the *Piestus* was strongly supported (ABS= 10; RBS= 57; BT= 98; PP= 1.00). The clade *Hypotelus* + *Eupiestus* was moderately supported (ABS= 8; RBS= 38; BT= 84; PP= 0.94) (Table 3).

Table 3 summarizes the results of the main topologies shown in the text, e. g., strict consensus tree from EW, IW tree (*K5*), and BI tree. Besides, it indicates if the clade was recovered as monophyletic in each analysis employed, as well as, the value of the branch support for each clade.

Table 3. Summary of the main clades recovered by the phylogenetic analyses of the subfamily Piestinae using adult data.

	Analysis							
	EW			IW			BI	
Clade	recovered clade	ABS	BT	recovered clade	RBS	BT	recovered clade	PP
Oxyteline Group	Yes	4	68	Yes	43	73	Yes	0.97
Scaphidiinae + Oxytelinae	No	–	–	Yes	13	–	No	–
Scaphidiinae	Yes	16	100	Yes	60	99	Yes	1.00
Oxytelinae	Yes	4	–	Yes	24	53	No	–
Osoriinae + Scaphidiinae	Yes	2	–	No	–	–	No	–
Osoriinae + Piestinae	No	–	–	Yes	13	–	Yes	0.68
Osoriinae	No	–	–	Yes	13	–	No	–
Piestinae	Yes	13	94	Yes	61	98	Yes	1.00
Prognathoides + (remain genera)	No	–	–	Yes	61	98	No	–
Prognathoides + (*Siagonium* + *Piestoneus*)	No	–	–	No	–	–	Yes	0.58
Piestus + (*Hypotelus* + *Eupiestus*)	Yes	3	–	Yes	17	–	Yes	0.65
Siagonium + *Piestoneus*	Yes	5	58	Yes	40	68	Yes	0.60
Hypotelus + *Eupiestus*	Yes	8	86	Yes	38	84	Yes	0.94
Piestus	Yes	10	98	Yes	57	98	Yes	1.00
Hypotelus	Yes	5	90	Yes	55	94	Yes	0.54
Eupiestus	Yes	15	99	Yes	54	99	Yes	1.00
Siagonium	No	–	–	No	–	–	No	–
Piestoneus	Yes	6	96	Yes	45	97	Yes	0.99

EW – Equal Weighs; IW – Implied Weights; BI – Bayesian Inference; ABS – Absolut Bremer Support; RBS – Relative Bremer Support; BT – Bootstrap; PP – Posterior Probability.

Table 4. List of transformations that support the main clades of the Oxyteline Group implied weights analysis, comparing with equal weights to the characters.

Clade	Transformations (IW)	Synapomorphic/ Homoplastic (IW)	EW: present (P) or absent (A)	Shared by all lineages: yes (Y), no (N)
Oxyteline Group	Antennal insertion concealed under a ridge or a shelf-like elevation at sides of frons (character 9:0)	Synapomorphic	P	Y
	Antennomere 3 evidently longer than antennomere 2 (character 12:2)	Homoplastic	A	N
	Prosternum, middle not prominent in relation to sides (character 63:0)	Synapomorphic	P	N
	Elytral epipleural ridge present (character 74)	Synapomorphy	P	N
	If spine present, base fused to the elytrum, curved (character 80:0)	Synapomorphic	P	N
	If spine present, apex not distinct, gradually fused to the elytrum (character 81:1)	Synapomorphic	A	N
	Mesothorax, presence of transverse mesepisternal carina, even if incomplete (character 90:1)	Synapomorphic	A	N
	Metathorax, mesocoxae cavities without transversal sclerite (character 108:0)	Synapomorphic	P	N
	Tergum II, anterior transverse subbasal carina absent or incomplete, not reaching the sides of the tergum (character 129:0)	Homoplastic	A	N
	Sterna II–III, metacoxal excavations present (character 138:1)	Synapomorphic	A	N
	Abdominal segment VII with one pair of paratergites (character 147:0)	Synapomorphic	A	N
	Tergum VIII, laterotergites indistinctly demarcated from the tergum VIII by fold (character 152:0)	Synapomorphic	P	N
Scaphidiinae + Oxytelinae	Epistomal (=frontoclypeal) suture present (character 6:1)	Synapomorphic	A	N
	Submentum and gula separated by internal ridge anterior to posterior tentorial pits (character 44:0)	Homoplastic	A	Y
	Gular sutures separate throughout (character 45:0)	Homoplastic	A	Y
	Mesothorax, mesoventrite process in relation to the metaventrite process, equal in length (character 100:1)	Homoplastic	A	N
	Metathorax, metaventrite process in relation to the mesoventrite process, apex equal or narrower (character 104:0)	Homoplastic	A	N
	Metathorax, metendoesternite, furcal arms not divided (character 114:0)	Homoplastic	A	N
Scaphidiinae	Posterior part of the head capsule, lateral constriction absent (character 0:0)	Homoplastic	A	Y
	Ommatidia structure, facets hexagonal and flat, eye surface smooth (character 8:0)	Homoplastic	P	Y
	Labrum not bilobed (character 16:0)	Homoplastic	P	Y
	Mandibles, mandibular apices in apposition concealed beneath labrum (character 17:0)	Synapomorphic	P	Y
	Labium, palpomere 2 wider than long (character 32:1)	Homoplastic	A	Y
	Mentum subquadrate or elongate (character 40:0)	Homoplastic	A	Y
	Gular sutures (or suture, if single), incomplete, obsolete in anterior 1/3 or 1/2 (character 46:0)	Homoplastic	A	Y
	Pronotum, furcasternum, two mesially endoskeleton, distance between the endoskeletons are longer than length of the endoskeleton (character 54:1)	Homoplastic	A	Y
	Elytra long covering most of tergal abdominal segments (character 67:0)	Synapomorphic	P	Y
	Elytra, humeral angle quadrate (character 79:1)	Homoplastic	P	Y
	Wing, anal lobe, additional lobule absent (character 86:0)	Homoplastic	A	Y
	Mesothorax modified with anterior excavation for each procoxae (character 92:1)	Synapomorphic	P	Y
	Mid-leg, mesotrochantin concealed (character 94:0)	Homoplastic	A	Y

Table 4. Continue

	Mid-leg, mesocoxae widely separated by at least half of coxal width (character 95:2)	Homoplastic	P	Y
	Metathorax, metaventrive, mesal posterior lobes present (character 111:1)	Synapomorphic	P	Y
	Thorax, alacrista straight vertically (character 116:0)	Homoplastic	A	Y
	Anterior leg, procoxae with deep groove and carina (character 121:1)	Homoplastic	A	Y
	Tergum III, anterior transverse subbasal carina absent (character 130)	Homoplastic	A	Y
	Terga IV–VII, anterior transverse subbasal carina absent (character 131:0)	Homoplastic	A	Y
	Sternum III, presence of lateral longitudinal carina on each side (character 134:1)	Homoplastic	A	Y
	Tergum II, spiracles inserted in the membrane, at edges of tergum II (character 135:0)	Homoplastic	P	Y
	Tergum VIII, absence of laterotergires (character 151:0)	Synapomorphic	P	Y
	Tergum IX male, apex of ventral struts fused each other (character 161:1)	Synapomorphic	A	Y
Oxytelinae	Antenna, antennomere 1 evidently longer than antennomeres 2–3 combined (character 11:2)	Homoplastic	A	N
	Mandible, complete transversal sulcus through molar region present (character 20:1)	Synapomorphic	P	Y
	Elytra, elytral striation, sutural stria absent (character 68:0)	Homoplastic	P	N
	Metathorax, contiguous mesocoxae cavities at the metasternite region (character 110:0)	Homoplastic	P	N
	Leg, empodial setae or setiform process absent (character 123:0)	Homoplastic	A	N
	Abdominal segment II, presence of paratergites (character 142:1)	Synapomorphic	P	Y
	Abdominal apex, defensive (odoriferous) glands in the terminalia (character 154:1)	Synapomorphic	P	Y
Osoriinae + Piestinae	Antenna, presence of setigerous protuberances on antennomeres, even if reduced (character 14:1)	Homoplastic	P	N
	Pronotum, anterior margin wider in relation to posterior margin (character 50:2)	Homoplastic	A	N
	Pronotum, front angles, relative to anterior margin of pronotum produced antirad (character 51:1)	Homoplastic	P	N
	Pronotum, internal mid-longitudinal ridge or projection, present, half of pronotal length or longer (character 52:2)	Homoplastic	P	N
	Elytra, apical inner margin, presence of small projection of elytral suture(character 77:1)	Synapomorphic	P	N
	Mesothorax, mesendosternites, shape, one pair of vertical stick and one projection curved posterad (character 96:3)	Synapomorphic	P	N
	Sterna II–III, intercoxal carina or elevation present (character 137:1)	Homoplastic	A	Y
Osoriinae	Posterior margin of head, occiput with a slightly or not projection at the mid (character 1:0)	Homoplastic	P	N
	Antenna, antennomere 1 length ratio to antennomeres 2–3 combined, evidently longer (character 11:2)	Homoplastic	A	N
	Antenna, antennal macrosetae present only on apical half of antennomeres (character 15:0)	Homoplastic	P	N
	Ligula, enlarged macrosetae near of the median sclerite (character 38:1)	Homoplastic	P	N
	Pronotum, internal anterior transversal carina (if present) sinuous-shaped (character 60:1)	Synapomorphic	P	N
	Tergum II, straight anterior margin at the middle (character 136:0)	Homoplastic	P	N
	Abdominal segments III-VI, absence of paratergites (character 144:0)	Synapomorphic	P	Y
	Abdominal segment VII, absence of paratergites (character 146:0)	Homoplastic	P	Y
	Tergum IX male, absence of ventral struts (character 159:0)	Homoplastic	P	N

Table 4. Continue

Piestinae	Dorsal tentorium arm, impression like reniform shape (character 4:1)	Homoplastic	P	N
	Eyes with cuticular projection of the head over the eye (character 7:1)	Homoplastic	P	N
	Labium, palpomere 1, small pit near the seta of the apical margin present (character 31:1)	Homoplastic	P	N
	Labium, palpomere 2 wider than long (character 32:2)	Homoplastic	A	N
	Cervical sclerites, insertion in the thorax, bilobed apex (character 49:1)	Synapomorphic	P	N
	Pronotum, prosternum, presence of internal oval posterior impression (character 57:1)	Homoplastic	P	N
	Elytra, sutural stria punctate and striate (character 69:2)	Synapomorphic	P	N
	Elytra, elytral striation, if sutural stria present, not reaching the marginal apex of elytra (character 70:0)	Homoplastic	P	N
	Elytra, elytral striation, non-sutural striae present (character 72:1)	Homoplastic	P	N
	Elytra, posterior margin keeled (character 76:1)	Synapomorphic	P	Y
	Wing, vein Cu, apex closer to the apex of the wing, in a straight line (character 89:1)	Homoplastic	A	N
	Anterior leg, procoxae with deep groove and carina (character 121:1)	Homoplastic	P	Y
	Tergum IX dorsally slightly divided by tergum X, distance between margins more or less gradually increasing posteriorly (character 156:0)	Synapomorphic	A	Y
	Tergum X, basal half, shape triangulate with sides emarginated (character 158:2)	Synapomorphic	P	N
	Tergum IX male, ventral struts short and not so projected (character 160:0)	Synapomorphic	P	Y
	Sternum IX male, base, shape pointed (character 162:0)	Homoplastic	P	Y
Piestus + (*Hypotelus* +*Eupiestus*)	Pronotum, furcasternum, two mesially endoskeletons, distance between the endoskeletons are longer than the length of the endoskeleton (character 54:1)	Homoplastic	P	N
	Pronotum, prosternum, prominent internal anterior transversal carina (character 59:1)	Homoplastic	P	Y
	Pronotum, internal transversal carina directed to the middle of prosternum (character 61:1)	Homoplastic	P	Y
	Wing, vein A1+A2, division occurs on the apical third (character 88:1)	Synapomorphic	P	Y
	Metathorax, presence of transversal sclerite in the mesocoxae cavities (character 108:1)	Homoplastic	P	Y
	Metathorax, metendoesternite, median process present (character 113:1)	Homoplastic	P	Y
	Hind leg, metacoxae, presence of evident median transversal sulcus (character 122:1)	Homoplastic	P	Y
	Tergum II, anterior transverse subbasal carina complete reaching sides of the tergum (character 129:1)	Homoplastic	A	N
Siagonium + *Piestoneus*	Maxilla, maxillary palpomere 4, presence of impressions near apex (character 24:1)	Homoplastic	P	Y
	Ligula, median sclerite, broad apical region (character 36:1)	Homoplastic	P	N
	Gular plate, presence of deep depression at the middle (character 47:1)	Homoplastic	P	Y
	Sterna II–III, absence of transverse carina in the metacoxal excavations (character 139:0)	Homoplastic	P	Y

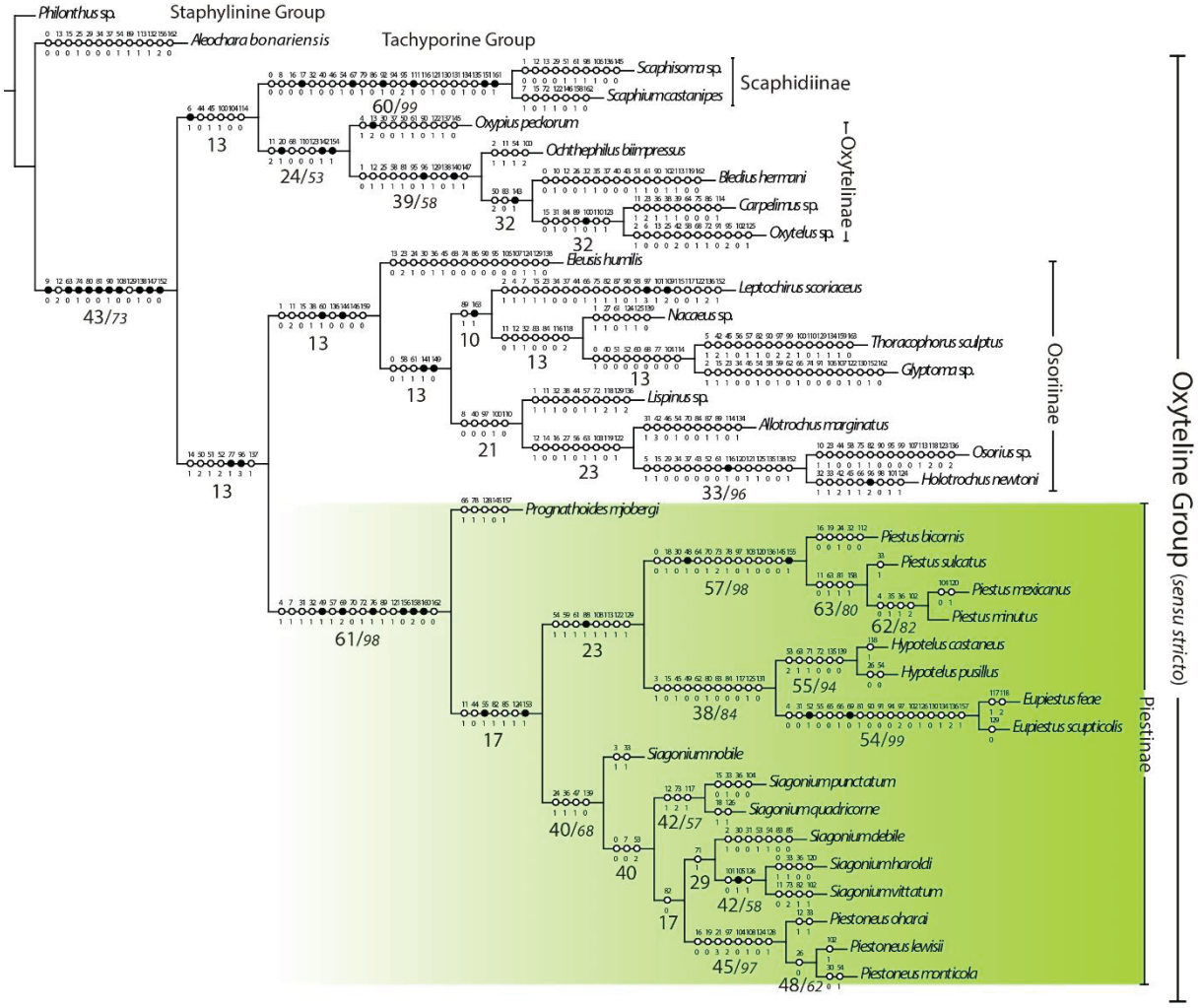

Figure 7. Reference topology – Implied weights analysis (adult). Black circles indicate synapomorphic transformations and white circles indicate homoplastic transformations. The character number is shown above the circle and the corresponding character state is shown below it. The number displayed under each branch corresponds to the value of relative Bremer support and *Bootstrap*.

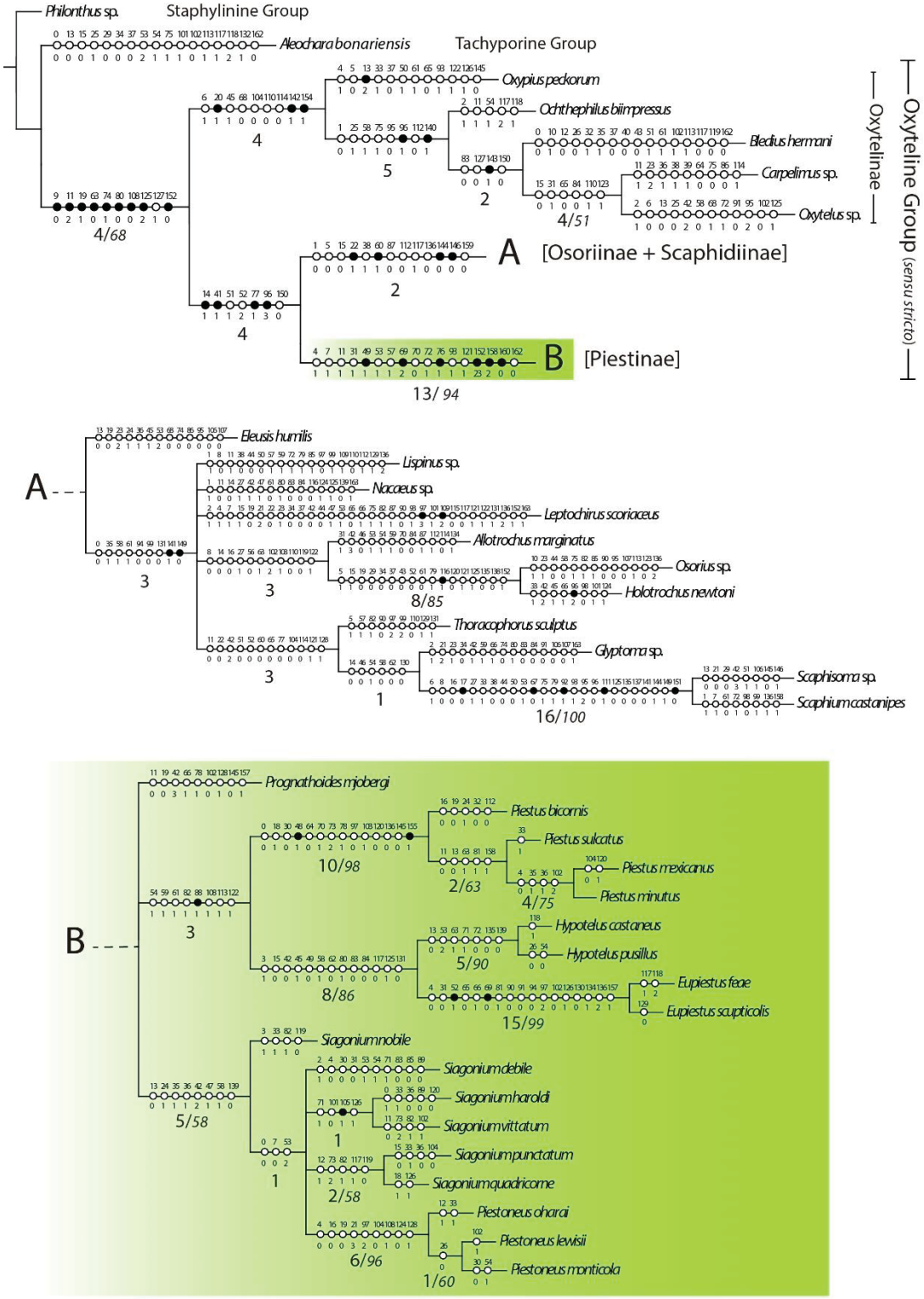

Figure 8. Equal weights analysis (adult) – strict consensus tree of the four most parsimonious trees obtained. Black circles indicate synapomorphic transformations and white circles indicate homoplastic transformations. The character number is shown above the circle and the corresponding character state is shown below it. The number displayed under each branch corresponds to the value of absolute Bremer support and *Bootstrap*.

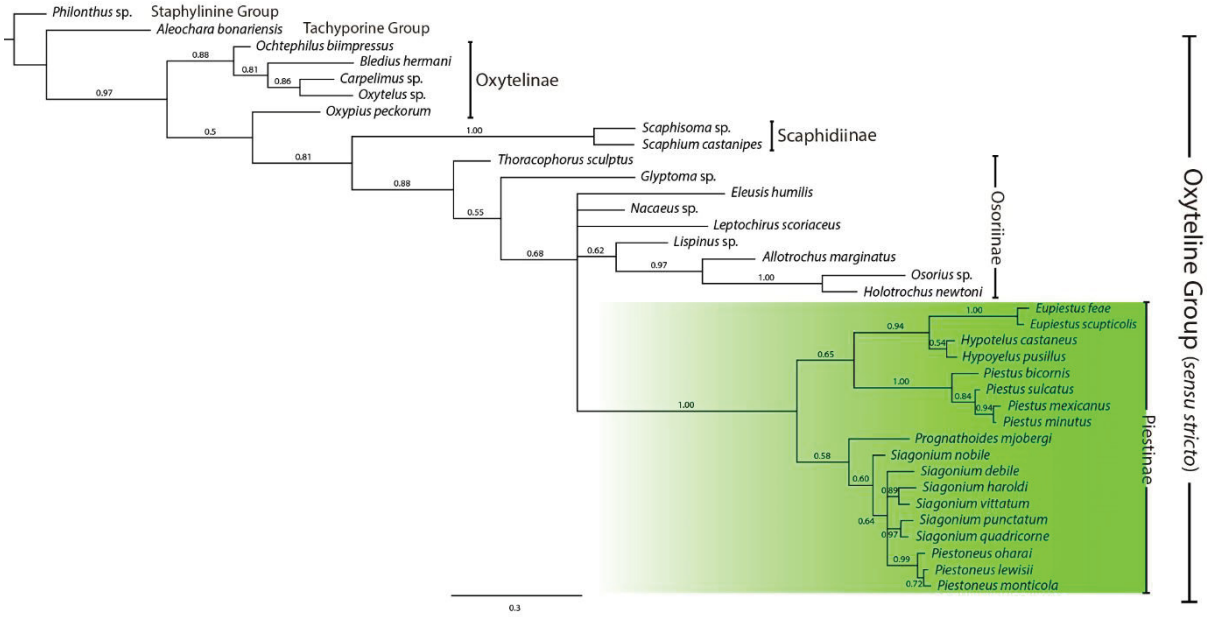

Figure 9. Majority rule (50%) consensus tree from the Bayesian analysis (adult). The number displayed above each branch corresponds to the value of the posterior probabilities.

4.3. DISCUSSION

4.3.1. Osoriinae, the sister-group of the Piestinae

Osoriinae is a huge group of 110 genera and more than 2,200 described species (Thayer 2016). They are the most “tropical” group of the most diverse subfamilies of Staphylinidae, living under bark of decaying logs, exhibiting saprophagous habits (Navarrete-Heredia *et al*. 2001).

The main topology (IW) recovered the monophyly of Osoriinae (Fig. 7), but the internal relationships of the groups were not recovered in agreement with Grebennikov & Newton (2012). Mckenna *et al*. (2015a) found Osoriinae as non-monophyletic based on molecular dataset, as well as our result under BI, which the clade was recovered as polyphyletic (Fig. 9).

Although our analyses differ on the monophyly of the subfamily Osoriinae (*e.g.*, EW and BI) our results allowed the stating of the Osoriinae, at least in part, as sister-group of the subfamily Piestinae.

4.3.2. Monophyly and composition of the Piestinae lineage

Even the new concept to Piestinae proposed by Newton & Thayer (1992) and the taxonomic changes (see Herman 2001 for details), this subfamily still remained not well-defined. Absence of apomorphies and/or similarity with related groups (Caron *et al*. 2012; Grebennikov & Newton 2012) and no clear phylogenetic results (Thayer 2016) have been contributing to that.

The Piestinae may indeed form a non-monophyletic group, in which the Osoriinae + some Piestinae together form a monophyletic group (Thayer 2016; S. -I. Naomi, personal communication). In other words, the genera of the Oxyteline Group may not be appropriately grouped into defined subfamilies because it is a basal group of the family Staphylinidae.

Previous studies such as Thayer (2005) (revision of literature), Grebennikov & Newton (2012) (phylogenetic analysis based on morphological evidences), Mckenna *et al*. (2015a) (phylogenetic analysis based on molecular evidences) have been shown Piestinae as a non-monophyletic group. Bortoluzzi *et al*. (2017), including four genera of piestines, agreed with Grebennikov & Newton (2012), even their focus was on testing the monophyly of the genus *Hypotelus*.

Grebennikov & Newton (2012) was the most consistent among all above-mentioned studies. Based on larval and adult morphological characters, the authors indicate the piestines are included in the clade comprised of the strict sense of the Oxyteline Group of subfamilies and they formed a basal grade with respect to Osoriinae, although the authors did not officially confirm the dissolution of the subfamily. Even using only one species per genus, they used the major number of taxa of the subfamily (until now). However, Piestinae studies always required more extensive taxon sampling and better resolution.

Notably, our results consistently supported the monophyly of Piestinae and their lineages, except to the genus *Siagonium*, which is paraphyletic without the inclusion of species of *Piestoneus*. The phylogenetic structure of the subfamily is the following: *Prognathoides* + (*Piestus* + (*Hypotelus* + *Eupiestus*)) + ("*Siagonium*" + *Piestoneus*). Therefore, our results do not corroborate the previous phylogenetic conclusions about this group.

Caron *et al*. (2012) focusing on to test the monophyly of the genus *Piestus* included all seven extant genera of Piestinae. They found a closer relationship

between *Hypotelus* + *Eupiestus*, as well as for *Siagonium* + *Parasiagonum* + *Piestoneus* + *Prognathoides*, but is important to be noted that they did not use out-group members out of the subfamily and their study was not designed to test the monophyly of the subfamily.

4.3.2.1. Basis of the Piestinae

Surprisingly, our results (IW) recovered *Prognathoides* as basal clade inside the subfamily and not closely related with *Siagonium* species. The basal position of the genus might be explained by the absence of the small sclerite above of paratergites at the abdominal segment III (153:0), present in all other piestines taxa herein studied.

Caron *et al*. (2012) found *Prognathoides* in a clade together with *Siagonium*, *Parasiagonum* and *Piestoneus* in their EW analysis, in contrast with the topology recovered in their IW analysis, which a closer relationship between *Prognathoides* and *Piestus*. Similar result was found by Grebennikov & Newton (2012). Their results are in contrast with our reference topology, but it is in similarity with our not supported branch results in the BI topology and one of most parsimonious trees under EW.

The basis of the Piestinae, and consequently the position of *Prognathoides*, remains unclear, once our analyses did not find congruence in the results and the previous results in the literature are not in congruence too. Two possible scenarios might be presented: (1) *Prognathoides* stay on the basis of the subfamily, being the sister-group of all piestines beetles; (2) *Prognathoides* forms a clade with "*Siagonium"* + *Piestoneus*. The possible inclusion of the genus *Parasiagonum* in further analyses might elucidate this issue.

4.3.2.2. *Eupiestus* and the Neotropical genera

Piestus and *Hypotelus* represent the lineages that are nearly restricted to the Neotropical region in a strict sense (Caron *et al*. 2012; Bortoluzzi *et al*. 2017). It was expected that these genera showed a closer relationship, but this has not been identified in any analysis presented here, in which *Hypotelus* is closer to *Eupiestus* (Palearctic and Oriental regions) instead.

Eupiestus are rare in collections (Rougemont 2014) and information about biology, habitus and/or aedeagus are poorly known in the literature (Yin & Li 2016).

They have been representing a challenge on evolutionary studies and it was expected less closely related to remaining genera (A.F. Newton, personal communication). Whereas in our results we recovered *Hypotelus* + *Eupiestus* as sister clade. Grebennikov & Newton (2012) recovered *Eupiestus* a basal group formed by "Piestinae" + Osoriinae + Oxytelinae. Caron *et al*. (2012) identified, even not well resolved, a closer relationship between *Hypotelus* and *Eupiestus*, although it is not possible to infer more precisely about this association because they used to root *Hypotelus* and restrict a number of terminals.

Following Bortoluzzi *et al*. (2017), *Hypotelus* consist a monophyletic group and more related to Osoriinae species than to Piestinae. This systematic result agrees with Grebennikov & Newton (2012) but is in contrast with the current work, which consistently recovered a monophylum of *Hypotelus* + *Eupiestus*. However, Bortoluzzi *et* al. (2017) did not include members of *Eupiestus* in their study and given that Osoriinae is a huge group of more than 100 genera, while Bortoluzzi *et al*. (2017) and that of Grebennikov & Newton (2012) included only one or seven genera of this group, respectively, premature results which infer about the relationship of *Hypotelus* to Osoriinae may have been found, likely.

4.3.2.3. Non-monophyly of *Siagonium*

The genus *Siagonium* is the second most species-rich genus of piestines (currently comprises 25 species), forming a homogenous group (Steel 1950) after the removal of the Australian and New Zeeland species (now in monotypic genera *Prognathoides* and *Parasiagonum*). Nonetheless, this genus appears to represent a polyphyletic clade in relation to the monophyletic genus *Piestoneus*, results supported by all analyses in this work.

Siagonium was found as sister to Oxytelinae by Grebennikov & Newton 2012, while Mckenna *et al*. (2015a) found the genus as sister-group of the clade *Eupiestus* + Osoriinae in part (Bayesian) or as sister-group to *Eupiestus* (maximum likelihood).

Even though all of our topologies indicate the systematic placement of *Piestoneus*, plus the non-recognition of the monophyly of *Siagonium*, we are not able to establish a new combination for these genera, once, originally, our study does not has the goal to test independently the monophyly of each genus of the subfamily. Thus, further studies focusing on this goal may confirm these results.

4.3.3. Conflicts over adult morphologic characters to delimiting Piestinae

The subfamily has been historically ill-defined and problematic, but even with several recent studies (e.g. Caron *et al*. 2012; Grebennikov & Newton 2012; Bortoluzzi *et al*. 2017) its concept is still based on plesiomorphic characters. This suggests that some morphological characters should be re-evaluated for characterizing the group.

Considering the currently available hypothesis in the literature and based on the topology recovered in IW approach, our results did not corroborate with the morphological taxonomic concept of Newton *et al*. (2000) and Navarrete-Heredia *et al*. (2002). Piestinae was characterized by having body relatively elongate and depressed; antennae inserted under shelf-like corners of frons; procoxae small, globose; protrochantin exposed; abdomen long and parallel-sided, with six visible sterna and one or two pairs of paratergites per segment; tarsi 5–5–5. The subfamily Piestinae was recovered as monophyletic in our study, but the analysis, did not recover any of those characters as synapomorphies. Based on the IW (Fig. 7), we recovered the following non-homoplastic synapomorphies (Table 4): bilobed apex of the cervical sclerites – 49:1 (Fig. 4A); punctate and striate sutural stria – 69:2; Elytra, posterior margin keeled –76:1; Tergum IX dorsally slightly divided by tergum X with distance between margins more or less gradually increasing posteriorly – 156:0; triangulate basal half of the tergum X – 158:2; and short ventral struts – 160:0. However, is possible to identify that just the synapomorphies 76:1, 156:0 and 160:0 are present on all lineages (Table 4).

The character 76 (Fig. 6F) is not well visible before dissection, being necessary to remove the elytrum from the specimens, but none chemical process of dissection is necessary. Another important characteristic observed in all piestines taxa studied here corresponds to the posterior external angle of elytra, which are lighter (Fig. 5F). This characteristic was not included in our matrix because we understand that it is corresponding to the transformation 76:1. This characteristic becomes more evident after soaking in 10% solution of KOH. Also is important to mention that the character transformation 156:0 is not exclusive to Piestinae, it has already identified in other members of the Staphylinine Group (Solodovnikov & Newton 2005).

The genus *Piestus* was recovered as monophyletic by Caron *et al*. (2012) and, at that time, it was established by 11 synapomorphies. The concept of *Piestus* was recently revised by Yamamoto *et al*. (2017) after the discovery of the species-fossil *Propiestus archaicus*. Its new concept includes all characters pointed out by

Yamamoto *et al*. (2017), and some others which may be re-evaluated to define the extant genera of Piestinae. The evident median carina on the mesoventrite process (103:1) was removed because it is present in the fossil and some *Siagonium* (Yamamoto *et al*. 2017), but it is applicable to define the extant genus *Piestus*. It is true that some *Siagonium* have the longitudinally carinate mesoventrite process, but it is much shorter and more discreet when comparing it to those of *Piestus*. Besides, openings of glands at the external margin of mandibles (character 22:1 in Caron *et al*. 2012) was indicated as present in *Siagonium* (*Siagonium quadricorne*) by Grebennikov & Newton (2012) and confirmed here, however, there is a difference between the *Piestus* and *Siagonium* mandibular cavities, while *S. quadricorne* have a single pit and more ventrally, it is on lateral surface and digitiform in *Piestus* (Figs 114–115 in Caron *et al*. 2012), as pointed out by Grebennikov & Newton (2012). Crowson & Ellis (1968) commented about the presence of the single small cavities opening on the mandibles in *P. mjobergi*, however, we did not find the structures during the MEV image analysis process. It is worth mentioning that Grebennikov & Newton (2012) scored, for *P. mjobergi*, as absent the presence of mandibular cavities, but we do not know if they did not see the structure, as well, or if the state of character has been scored as "absent" due to the wording character.

The genus *Hypotelus*, recently recovered as monophyletic by Bortoluzzi *et al*. (2017), was supported by eighth synapomorphies. Based on the current work, some characteristics were not confirmed and others were better explored, thus, *Hypotelus* may be defined by the: presence of two pairs of macrosetae near the median sclerite of the ligula (Caron *et al*. 2012; Bortoluzzi *et al*. 2017). This character is present on all other piestines taxa studied here, but it seems that occurs only one pair instead. Moreover, *Hypotelus* have non-sutural striae on elytra (Caron *et al*. 2012; Grebennikov & Newton 2012; Bortoluzzi *et al*. 2017) and the antennomeres 5–11 densely covered with microsetae (Bortoluzzi *et al*. 2017). All piestines genera have the antennomeres covered with setae (Fig. 6A–C), but only *Hypotelus* (antennomeres 5–11) and *Piestus* (antennomeres 4–11) have the densely covered pattern of microsetae described by Caron *et al*. (2012) and Bortoluzzi *et al*. (2017) (in the other genera, the microsetae are sparser and are present in other combination of antennomeres). Another diagnostic characteristic is the apex of prosternal process easily visible behind the procoxae (Bortoluzzi *et al*. 2017). *Prognathoides* also have the apex of prosternal process projected behind the procoxae, but they differ from each other, while the

prosternum is curved projecting posteriorly dorsad to procoxae and visible again behind them in *Hypotelus*, it is straight and visible between procoxae throughout its all length in *Prognathoides*.

5. PART II – MONOPHYLY OF PIESTINAE BASED ON LARVAL MORPHOLOGICAL DATA

5.1. MATERIAL AND METHODS

5.1.1. Examined material

We received the specimens previously identified by institutions, and their identifications were adopted as correct. The material studied (Appendix 5) belongs to the following collections: Coleção Entomologica Pe. J. S. Moure, Universidade Federal do Paraná, Curitiba (DZUP, Lúcia Massutti de Almeida); Field Museum Natural History, Chicago (FMNH, Crystal Maier); Museu de Zoologia da Universidade de São Paulo, São Paulo, (MZUSP, Sônia Aparecida Casari).

This study was conducted at the Laboratório de Pesquisa em Coleoptera (LAPCol), Departamento de Biodiversidade, Universidade Federal do Paraná–Setor Palotina, Palotina, Paraná, Brasil.

5.1.2. Choice of in-group and out-group taxa

The larval morphological data were scored, in most of the cases, for the same species (compare Table 2 and Table 5; see Fig. 10). In cases where this was impossible because of material limitation, different congeneric species were used. Even though in larvae an exact determination of the instar is not always possible, we believe that only older-instar larvae were used to score characters, and we assume that comparing characters is legitimate even if possibly scored for different older instars. Some larvae used in this work corresponding to the same material used by Grebennikov & Newton (2012).

The choices of larval terminals follow the same parameters mentioned for adult morphological data (see subitem 4.1.2. Choice of in-group and out-group taxa, in Part I)..

Table 5. List of 22 larval terminals included in the phylogenetic analysis to detect the monophyly of the subfamily Piestinae.

Family	Group	Subfamily	Tribe	Species
Out-group (15 spp.):				
Staphylinidae	Staphylinine	Staphylininae	n/a	*Philonthus* sp.
Staphylinidae	Tachyporine	Tachyporine	n/a	*Aleochara bonariensis*
Staphylinidae	Oxyteline	Scaphidiinae	Scaphisomatini	*Scaphisoma* sp.
Staphylinidae	Oxyteline	Scaphidiinae	Scaphidiini	*Scaphium castanipes*
Staphylinidae	Oxyteline	Oxytelinae	Bledini	*Bledius hermani*
Staphylinidae	Oxyteline	Oxytelinae	Oxytelini	*Ochthephilus biimpressus*
Staphylinidae	Oxyteline	Osoriinae	Eleusini	*Eleusis humilis*
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Allotrochus marginatus*
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Lispinus sp.*
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Nacaeus* sp.
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Thoracophorus sculptus*
Staphylinidae	Oxyteline	Osoriinae	Thoracophorini	*Glyptoma sp.*
Staphylinidae	Oxyteline	Osoriinae	Leptochirini	*Leptochirus scoriaceus*
Staphylinidae	Oxyteline	Osoriinae	Osoriini	*Osorius* sp.
Staphylinidae	Oxyteline	Osoriinae	Osoriini	*Holotrochus newtoni*
In-group (7 spp.):				
Staphylinidae	Oxyteline	Piestinae	n/a	*Eupiestus* sp.
Staphylinidae	Oxyteline	Piestinae	n/a	*Hypotelus pusillus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestus bicornis*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestus mexicanus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Piestus minutus*
Staphylinidae	Oxyteline	Piestinae	n/a	*Prognathoides mjobergi*
Staphylinidae	Oxyteline	Piestinae	n/a	*Siagonium punctatum*

5.1.3. Morphological terminology

The terminology and morphological interpretations adopted herein were based on Naomi (1987–1990), Costa & Ide (2006) and Grebennikov & Newton (2012). Several characters were adopted from Grebennikov & Newton (2012), then, their terminology was put on precedence. Few changes were made in the terminology to establish an agreement with that described for adults.

The measurements were given as the maximum length. Some diagnostic characters are shown on the figures using arrows.

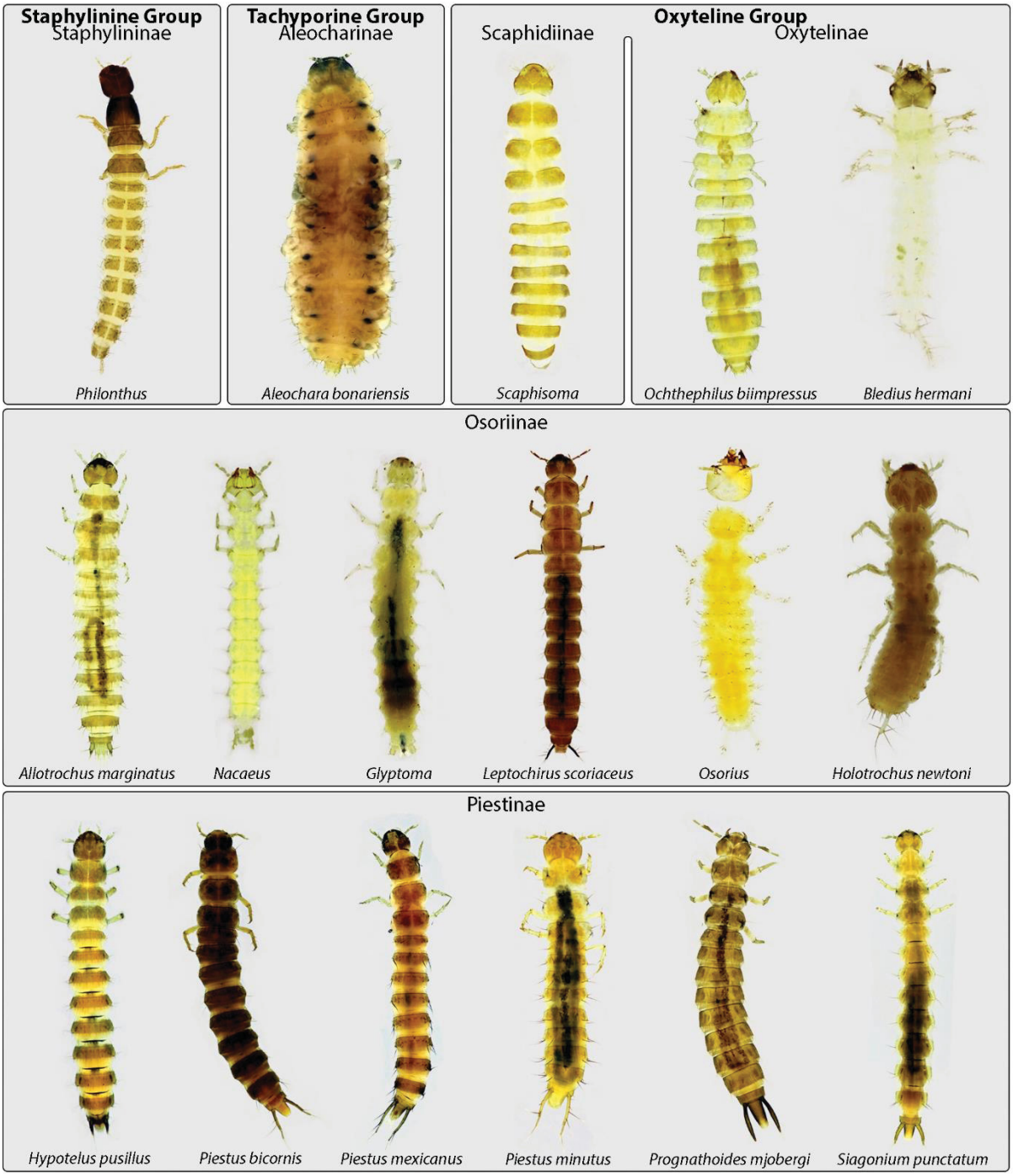

Figure 10. Larval habitus of some terminal taxa included in the phylogenetic analysis.

5.1.4. Preparation and illustration

Preparation of the larva specimens for the morphological study included macerating in hot 10% KOH solution for 1–2 minutes according to the body size and, for some specimens, subsequent staining with chlorazol black or ethyl green.

Specimens were mounted in glycerol on temporally microscope slides, allowing for free manipulation and rotation of the body parts. Some specimens were not dissected, allowing a better understanding of their morphological structures. After the study, specimens were mounted in Verniz Vitral on permanent microscope slides, or, for large specimens, stored in alcohol.

Habitus images were taken using a Canon EOS 80D camera in conjunction with a Canon MP-E 65mm f/2.8 1-5X Macro Lens. Images of the dissected parts were taken using a Canon EOS 80D in conjunction with a 10X Infinity Plan Achromatic Microscope Objective mounted on a Canon EF 28-135mm f/3.5-5.6 IS USM Lens. Image Fig.11F-detail was taken using a Moticam 5 CMOS digital camera attached on a Nikon Eclipse E200 light microscope. Scanning electron microscope imaging was taken with a Tescan Vega 3 at Laboratório de Microscopia Eletrônica de Varredura, UFPR.

Image stacks were processed using Helicon Focus version 6.7.1. Final editing was performed using Adobe Photoshop CC software, version 2015.0.0.

5.1.5. Phylogenetic analyses

The matrix was built in the program NEXUS Data Editor, version 0.5.0 (Page 2001) and comprise 22 terminal taxa, seven from in-group and 15 from out-group. Characters not observed were coded as '?' and those not applicable were coded as '-'.

The phylogenetic analyses were carried out using the same parameters for adult morphological data (see subitem 4.1.5. Cladistic analysis, in Part I).

5.2. RESULTS

5.2.1. Character list

A total of 74 characters were examined and coded, including external exoskeleton and endoskeleton. The following characters were scored as follows (Appendix 7):

Head:

0. *Head, width [dorsal view]*: (0) ≤ 0.8x width of prothorax; (1) 0.9–1.0x width of prothorax; (2) ≥ 1.1x width of prothorax. CI= 0.25, RI= 0.40. Character from Grebennikov & Newton (2012).
1. *Head, coronal suture* (Fig. 11A)*, extension from its starting point on posterior margin of head capsule, compared to total length of head capsule [dorsal view]*: (0) < 0.2x; (1) 0.3–0.5x; (2) > 0.5. CI= 0.40, RI= 0.57. Character from Grebennikov & Newton (2012).
2. *Head, hypostomal ridge*: (0) sinuous; (1) curved (Fig. 11I). CI= 0.50, RI= 0.75.
3. *Head, hypostomal ridge, posterior extension [ventral view]*: (0) not reaching posterior tentorial pits (Fig. 11I); (1) reaching posterior tentorial pits. CI= 0.25, RI= 0. Character from Grebennikov & Newton (2012).
4. *Head, posterior tentorial arms, posterior extension [ventral view]*: (0) absent; (1) present (Fig. 11F). CI= 0.33, RI= 0.50. Character modified from Grebennikov & Newton (2012).
5. *Head, posterior tentorial arms, posterior extension, anterior attachment [ventral view]*: (0) to the rest of tentorium (Fig. 11F); (1) to occipital rim and connected with the rest of tentorium; (2) to occipital rim and not connected with the rest of tentorium. CI= 0.50, RI= 0. Character modified from Grebennikov & Newton (2012).
6. *Head, corpotentorium (=Tentorial bridge) [ventral view]*: (0) absent; (1) present, even if extremely thin and thread-like. CI= 0.50, RI= 0.75. Character modified from Grebennikov & Newton (2012).
7. *Head, posterior tentorial pits [ventral view]*: (0) fused; (1) separated (Fig. 11F). CI= 0.50, RI= 0.85.
8. *Head, posterior tentorial pits, shape and number [ventral view]*: (0) two curved pits (Fig. 11F); (1) single transverse straight pit; (2) single vertical straight pit. CI= 0.40, RI= 0.70. States of character modified from Grebennikov & Newton (2012).
9. *Head, posterior pits, transverse distance between them [ventral view]*: (0) pits touching each other or forming a single pit; (1) not touching and distance not greater than width of widest maxillary palpomere (Fig. 11F); (2) distance greater than in state 1, but smaller than width of submentum. CI= 0.33, RI= 0.55. Character from Grebennikov & Newton (2012).

10. *Head, ventro-medial edges of epicranial plates, length [ventral view]*: (0) not longer than width of maxillary palpomere; (1) longer than state 0 and shorter than length of prementum; (2) subequal to or longer than length of prementum. CI= 0.25, RI= 0.25. Character from Grebennikov & Newton (2012).
11. *Head, stemmata, number [lateral view]*: (0) nil; (1) one; (2) two; (3) three; (4) four; (5) five. CI= 0.50, RI= 0.44. Character from Grebennikov & Newton (2012).
12. *Head, stemmata, size*: (0) small; (1) large (Fig. 11H). CI= 0.20, RI= 0.42.
13. *Head, clerotized strip separating dorsal mandibular articulation from antennal attachment [*dorsal view*]:* (0) very narrow and almost invisible; (1) wider, half width of antennomere 1; (2) subequal in width to antennomere 1. CI= 0.22, RI= 0.22. Character from Grebennikov & Newton (2012).
14. *Head, internal transverse ridge between anterior tentorial arm attachment and dorsal mandibular condyle, shape [dorsal view]*: (0) straight; (1) curved. CI= 0.25, RI= 0.25. Character from Grebennikov & Newton (2012).
15. *Head, internal transverse ridge between anterior tentorial arm attachment [dorsal view]*: (0) horizontally in most of its length; (1) evidently diagonally; (2) parallel. CI= 0.28, RI= 0.37.
16. *Head, antennae, length compared to length of head capsule [dorsal view]*: (0) < 0.5x; (1) 0.5–1.1x. CI= 0.33, RI= 0.33. Character modified from Grebennikov & Newton (2012).
17. *Head, antennae, penultimate antennomere, main sensory antennal appendage, its position with respect to articulation of apical antennomere [dorsal view]*: (0) anterior/mesal (Fig. 11D); (1) dorsal. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
18. *Head, antennae, penultimate antennomere, sensory antennal appendage, number*: (0) one, only sensory appendage (Fig. 11D); (1) two, sensory appendage and solenidium. CI= 0.16, RI= 0.16.
19. *Head, antennae, penultimate antennomere, long setae on the same position line of the main sensory antennal appendage, distance between them*: (0) shorter than length of main sensory antennal; (1) longer than length of main sensory antennal (Fig. 11D). CI= 0.16, RI= 0.37.
20. *Head, labrum/nasale, symmetry [dorsal view]*: (0) symmetrical; (1) asymmetrical, even if slightly. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).

21. *Head, labrum/nasale, anterior edge, main outline at middle (excluding teeth or serration) [dorsal view]*: (0) convex; (1) straight; (2) concave. CI= 0.40, RI= 0.50. Character from Grebennikov & Newton (2012).
22. *Head, labrum/nasale, median projection [dorsal view]*: (0) absent; (1) present. CI= 0.33, RI= 0.50. Character from Grebennikov & Newton (2012).
23. *Head, labrum, a pair of dark internal sclerites near of frontal margin [dorsal view]*: (0) absent; (1) present (Fig. 11G). CI= 0.50, RI= 0.88. Note: internal sclerites= endoskeleton/tentorium of labrum.
24. *Head, labrum, a pair of dark internal sclerites, if present, number and shape [dorsal view]*: (0) one pair of semicircular sclerites (Fig. 11G); (1) single sclerite of arch-shaped. CI= 1, RI= 1.
25. *Head, mandibles, apices, shape [dorsal view]*: (0) acutely pointed; (1) bifid, multifid or variously shaped, not acute. CI= 0.50, RI= 0.75. Character from Grebennikov & Newton (2012).
26. *Head, mandibles, apical quarter, maximal number of subapical teeth (excluding serration and the main mandibular apex) [dorsal view]*: (0) nil, subapical teeth absent; (1) one; (2) two; (3) three. CI= 0.33, RI= 0.25. Character from Grebennikov & Newton (2012).
27. *Head, mandibles, width at base compared to width at middle [dorsal view]*: (0) 1–1.5; (1) 1.7–3; (2) 5.0–7.0. CI= 0.33, RI= 0.77. Character from Grebennikov & Newton (2012).
28. *Head, mandibles, angle of medial outline between widened basal and narrowed apical parts [dorsal view]*: (0) absent; (1) present (Fig. 11C). CI=0.50; RI=0.85. Character modified from Grebennikov & Newton (2012).
29. *Head, mandibles, angle of medial outline between widened basal and narrowed apical parts, if present [dorsal view]*: (0) present, smooth or forming obtuse angle; (1) present, sharp, forming right angle (Fig. 11C). CI= 1, RI= 1. Character modified from Grebennikov & Newton (2012).
30. *Head, mandibles, retinaculo*: (0) absent; (1) present. CI= 0.20; RI= 0.42.
31. *Head, mandibles, symmetry [dorsal view]*: (0) symmetrical; (1) asymmetrical. CI= 0.25, RI= 0.40. Character modified from Grebennikov & Newton (2012).
32. *Head, malea and lacinia (or mala, if fused), shape [ventral view]*: (0) widest at the base; (1) widest distad of base. CI=0.20, RI=0.60. Character from Grebennikov & Newton (2012).

33. *Head, mala, bottom projected tooth [ventral view]*: (0) absent; (1) present (Fig. 11J). CI= 1, RI= 1.
34. *Head, mala, apparently articulated apical tooth [ventral view]*: (0) absent; (1) present. CI= 1, RI= 1. Note: this character was observed in microscope light compound, so, the apical tooth for the terminals scored as "1" looks like articulated (there is a lighter strip delimiting it), but in fact it is not.
35. *Head, mala, inner margin, serrated projections over the articulated teeth set [ventral view]*: (0) absent; (1) present (Fig. 11J). CI= 0.50, RI= 0.66.
36. *Head, mala, apex [ventral view]*: (0) modified in brush of setae; (1) not modified in brush of setae. CI= 50; RI= 0.
37. *Head, mala, outer margin, projection [ventral view]*: (0) absent (Fig. 11E); (1) present. CI= 0.25, RI= 0.40.
38. *Head, maxilla, second (penultimate) maxillary palpomere, location of setae [ventral/dorsal view]*: (0) on apical third; (1) on apical half; (2) on all over palpomere. CI= 0.28, RI= 0.28.
39. *Head, maxilla, third (apical) maxillary palpomere, number of setae exceeding in length the width of palpomere [ventral/dorsal view]*: (0) nil; (1) one. CI= 0.50, RI= 0.50. Character from Grebennikov & Newton (2012).
40. *Head, maxilla, maxillary palpomere 3*: (0) shorter or equal palpomere 2; (1) longer than palpomere 2. CI= 0.14, RI= 0.33.
41. *Head, ligula [ventral view]*: (0) absent; (1) present. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
42. *Head, ligula, proportions [ventral view]*: (0) longer than wide; (1) about as long as wide; (2) wider than long. CI= 0.40, RI= 0.50. Character from Grebennikov & Newton (2012).
43. *Head, ligula, width at apex compared to width of basal labial palpomere [ventral view]*: (0) ≥ 1.5x; (1) ≤ 1x. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
44. *Head, ligula, apex, shape [ventral view]*: (0) pointed, rounded or straight, not bilobed at apex; (1) bilobed or tetralobed at apex (by a notch at middle). CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
45. *Head, prementum, ventral sclerite [ventral view]:* (0) entire; (1) longitudinally subdivided along midline by membrane. CI= 0.20, RI= 0. Character modified from Grebennikov & Newton (2012).

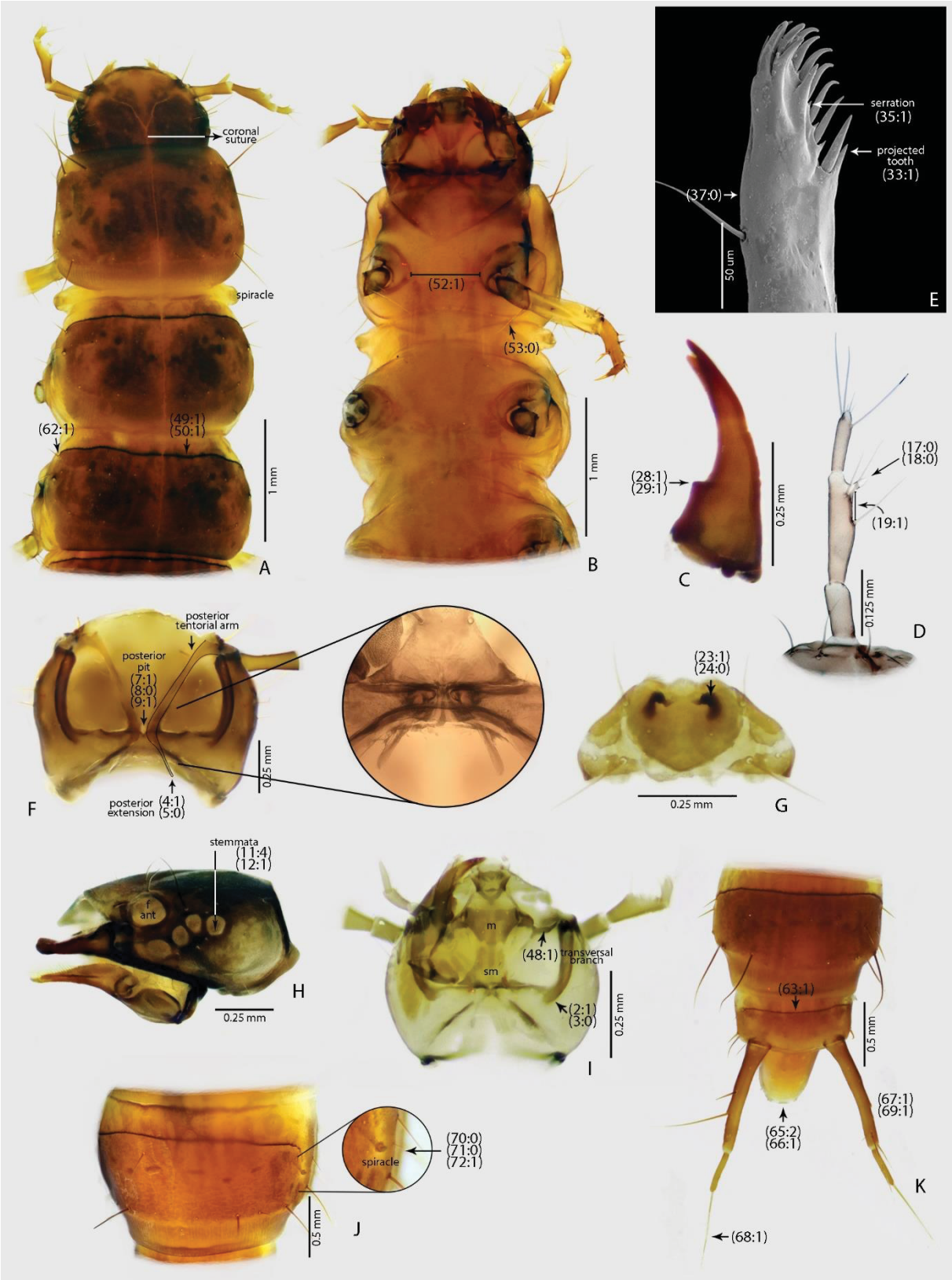

Figure 11. Larva morphological characters of some terminal taxa included in the phylogenetic analyses: head and prothorax. (A–C, F–I, K) *Piestus bicornis*; (D) *Prognathoides mjobergi*; (E) *Siagonium punctatum* (J) *Leptochirus scoriaceus*. (A,B) body, dorsal and ventral; (C) mandible; (D) antenna; (E) mala; (F, I) head, ventral; (G)

labrum, dorsal; (H) head, lateral; (J) abdominal segment VIII; (K) end of abdominal segments.

46. *Head, submentum, laterally, whether free from head capsule [ventral view]:* (0) free; (1) fused. CI= 0.50, RI= 0.50. Character modified from Grebennikov & Newton (2012).
47. *Head, mentum, basal sides, lobulal expansion [ventral view]*: (0) absent; (1) present. CI= 0.16, RI= 0.
48. *Head, transversal branch connecting the sides of the mentum to ventral articulation where the condyle of mandibles are inserted [ventral view]:* (0) branch weakly sclerotized; (1) branch strongly sclerotized, almost reaching the condyle connection (Fig. 11I). CI= 0.25, RI= 0.62.

Thorax:

49. *Meso and metathorax, anterior transverse subbasal carina [dorsal view]*: (0) absent; (1) present (Fig. 11A). CI= 0.33, RI= 0.71.
50. *Meso and metathorax, anterior transverse subbasal carina, if present [dorsal view]:* (0) incomplete; (1) complete (Fig. 11A). CI= 0.33, RI= 0.
51. *Transversal endoskeleton of the thorax [ventral view]*: (0) absent; (1) present. CI= 0.50, RI= 0.
52. *Procoxae, distance between them [ventral view]*: (0). Shorter than length of the protrochanter; (1) as long as/to one and half longer than length of the protrochanter (Fig. 11B); (2) two times longer than length of the protrochanter. CI= 0.22, RI= 0.
53. *Trochanter*, *longest seta, length [ventral view]*: (0) ≥ 2x longer than width of trochanter (Fig. 11B); (1) ≤ 1.5x longer than width of trochanter. RI= 0.20, RI= 0.42.
54. *Legs, tibiotarsus, setae, aspect*: (0) spine-like setae; (1) stout spine-like setae. CI= 0.14, RI= 0.14.
55. *Legs, tarsunguli, articulated spines, number on each tarsunguli*: (0) null; (1) one; (2) two; (3) three. CI= 0.50, RI= 0.25.
56. *Legs, tarsunguli, length*: (0) 3x longer than width of the tarsunguli; (1) 4x longer than width of the tarsunguli; (2) 5x longer than width of the tarsunguli. CI= 0.20, RI= 0.33.

Abdomen:

57. *Abdomen, shape [dorsal view]:* (0) evenly narrowing posterad; (1) parallel-sided most of its length; (2) widening posterad to about 1.2x of thoracic width. CI= 0.33, RI= 0.33. Character from Grebennikov & Newton (2012).
58. *Abdominal segments VII–IX*: (0) tergal plates equal in length than ventral plates; (1) tergal plates longer in length than ventral plates. CI= 1, RI= 1. Character from Grebennikov & Newton (2012).
59. *Abdominal terga I–VIII, one pair of incospicous setae inserted anterolaterad [dorsal view]*: (0) absent; (1) present. CI= 0.25, RI= 0.62.
60. *Abdominal segments, anterior transversal dark strip [dorsal and ventral view]*: (0) absent; (1) present. CI= 0.33, RI= 0.77.
61. *Abdominal segments I–VIII, anterior transverse subbasal carina [dorsal view]*: (0) absent; (1) present. CI= 1, RI= 1.
62. *Abdominal segments I–VIII, anterior transverse subbasal carina, if present, [dorsal view]*: (0) apical ending unobstructed, free; (1) apical ending obstructed by one seta (Fig. 11A). CI= 0.50, RI= 0.80.
63. *Abdominal segment X, anterior transverse subbasal carina [dorsal view]*: (0) absent; (1) present (Fig. 11K). CI= 0.50, RI= 0.87. Note: in *Leptochirus*, the anterior transverse subbasal carina is incomplete.
64. *Sternum IX, anterior transverse subbasal carina [ventral view]*: (0) absent; (1) present. CI= 0.50, RI= 0.85.
65. *Segment X (pygidium), shape [dorsal view]*: (0) distinctly wider than long; (1) about as wide as long; (2) distinctly longer than wide (Fig. 11K). CI= 0.50, RI= 0.60. Character from Grebennikov & Newton (2012).
66. S*egment X (pygidium), apical region [dorsal view]*: (0) with some long and evident setae; (1) with some inconspicuous setae (Fig. 11K). CI= 1, RI= 1.
67. *Urogomphi, number of segments [dorsal view]*: (0) one; (1) two (Fig. 11K). CI= 0.25, RI= 0.57. Character from Grebennikov & Newton (2012).
68. *Urogomphi, long apical seta (at least 25% of urogomphal length) [dorsal view]*: (0) absent; (1) present. CI= 0.25, RI= 0.40. Character from Grebennikov & Newton (2012).
69. *Urogomphi, length compared to length of tergum VIII [dorsal view]*: (0) < 1x; (1) 1–1.5x (Fig. 11K); (2) 2x. CI= 0.25, RI= 0.40. Character from Grebennikov & Newton (2012).

70. *Spiracle, localization*: (0) visible in dorsal view (Fig. 11J); (1) visible in lateral view. CI= 0.20, RI= 0.55.
71. *Spiracle, localization*: (0) in tergum, region totally sclerotized (Fig. 11J); (1) in tergum, region membranous or weakly sclerotized. CI= 0.16, RI= 0.44.
72. *Spiracle*: (0) protruded like a tube (1) not protruded (Fig. 11J). CI= 0.14, RI= 0.25.
73. *Elongated and looped gut*: (0) absent; (1) present. CI= 1, RI= 1. Character explained in Mckenna *et al*. (2015a).

5.2.2. Equal weighting versus implied weighting versus Bayesian analysis

Maximum parsimony (MP) analyses under equal weights (EW) returned two equally parsimonious trees with a length of 291 steps, with CI= 0.33 and RI= 0.53. The topology of the strict consensus is shown in Fig. 13.

The best topology attributing IW generated two trees with 300 steps and 27.044 adjusted fit (Appendix 6) and its consensus is shown in Fig. 12. The best topology corresponds to the *K5* criterion (K value equal to 3.545). RBS was calculated from 8,568 suboptimal trees and is shown below each clade branch.

The Bayesian inference (BI) analysis (Fig. 14) converged before 10 million generations and, at the end of the run, an average standard deviation of split frequencies had stabilized well below 0.005, while nearly all PSRF values were 1.000 (minimum = 1.000; maximum= 1.001).

All analyses consistently recovered the monophyly of the subfamily Piestinae, but just the BI recovered it with high support: EW with ABS= 6 and BT= 53; IW with RBS= 43 and BT= 57; and, BI with PP= 0.99 (Table 6). The clade is supported by nine common transformations, seven homoplastic [9:1; 10:0; 12:1; 13:2; 23:1; 28:1; 40:0] and two synapomorphic [33:1; 35:1] (Table 7; Fig. 12).

Table 6 summarizes the results of the main topologies shown in the text, e. g., strict consensus tree from EW, IW tree (*K5*), and BI tree. Besides, it indicates if the clade was recovered as monophyletic in each analysis employed, as well as, the value of the branch support for each clade.

Table 6. Summary of the main topologies of phylogenetic analyses of the subfamily Piestinae for larval data.

	Analysis							
	EW			IW			BI	
Clade	recovered clade	ABS	BT	recovered clade	RBS	BT	recovered clade	PP
Oxyteline Group	Yes	1	–	Yes	46	–	Yes	0.72
Scaphidiinae	Yes	4	–	Yes	17	–	Yes	0.87
Oxytelinae	No	–	–	No	–	–	Yes	0.72
Osoriinae	No	–	–	No	–	–	No	–
Piestinae	Yes	6	53	Yes	43	67	Yes	0.99

EW – Equal Weighs; IW – Implied Weights; BI – Bayesian Inference; ABS – Absolut Bremer Support; RBS – Relative Bremer Support; BT – Bootstrap; PP – Posterior Probability.

Table 7. List of transformations that support the main clades of the Oxyteline Group implied weights analysis, comparing with equal weights to the characters.

Clade	Transformations (IW)	Synapomorphic/ Homoplastic (IW)	EW: present (P) or absent (A)	Shared by all lineages: yes (Y), no (N)
Oxyteline Group	Antennae, length compared to length of head capsule, 0.5–1.1x. (character 16).	Synapomorphic	A	N
	Mandibles asymmetrical (character 31)	Synapomorphic	P	N
	Galea and lacinia (or mala, if fused) widest distad of base (character 32)	Homoplastic	A	N
	Ligula, width at apex compared to width of basal labial palpomere ≥ 1.5x (character 43)	Synapomorphy	P	N
	Elongated and looped gut present (character 73)	Synapomorphic	P	Y
Scaphidiinae	Single vertical straight posterior tentorial pit (character 8)	Homoplastic	A	Y
	Ventro-medial edges of epicranial plates not longer than width of maxillary palpomere (character 10)	Homoplastic	A	Y
	Five stemmata (character 11)	Synapomorphic	P	Y
	Curved internal transverse ridge between anterior tentorial arm attachment and dorsal mandibular condyle (character 14)	Homoplastic	A	Y
	Ligula absent (character 41)	Synapomorphic	P	Y
Piestinae	Posterior pits not touching each other and transverse distance between them not greater than width of widest maxillary palpomere (character 9)	Homoplastic	A	N
	Ventro-medial edges of epicranial plates not longer than width of maxillary palpomere (character 10)	Homoplastic	A	N
	Stemmata large (character 12)	Homoplastic	A	N
	Sclerotized strip separating dorsal mandibular articulation from antennal attachment, subequal in width to antennomere 1 (character 13)	Homoplastic	A	N
	Labrum, a pair of dark internal sclerites near of frontal margin, present (character 13)	Homoplastic	P	Y
	Mandibles, angle of medial outline between widened basal and narrowed apical parts, present (character 28)	Homoplastic	P	Y
	Mala, bottom projected tooth, present (character 33)	Synapomorphic	P	Y
	Mala, inner margin, serrated projections over the articulated teeth set, present (character 35)	Synapomorphic	P	N
	Maxilla, maxillary palpomere 3 shorter or equal than palpomere 2 (character 40)	Homoplastic	P	N

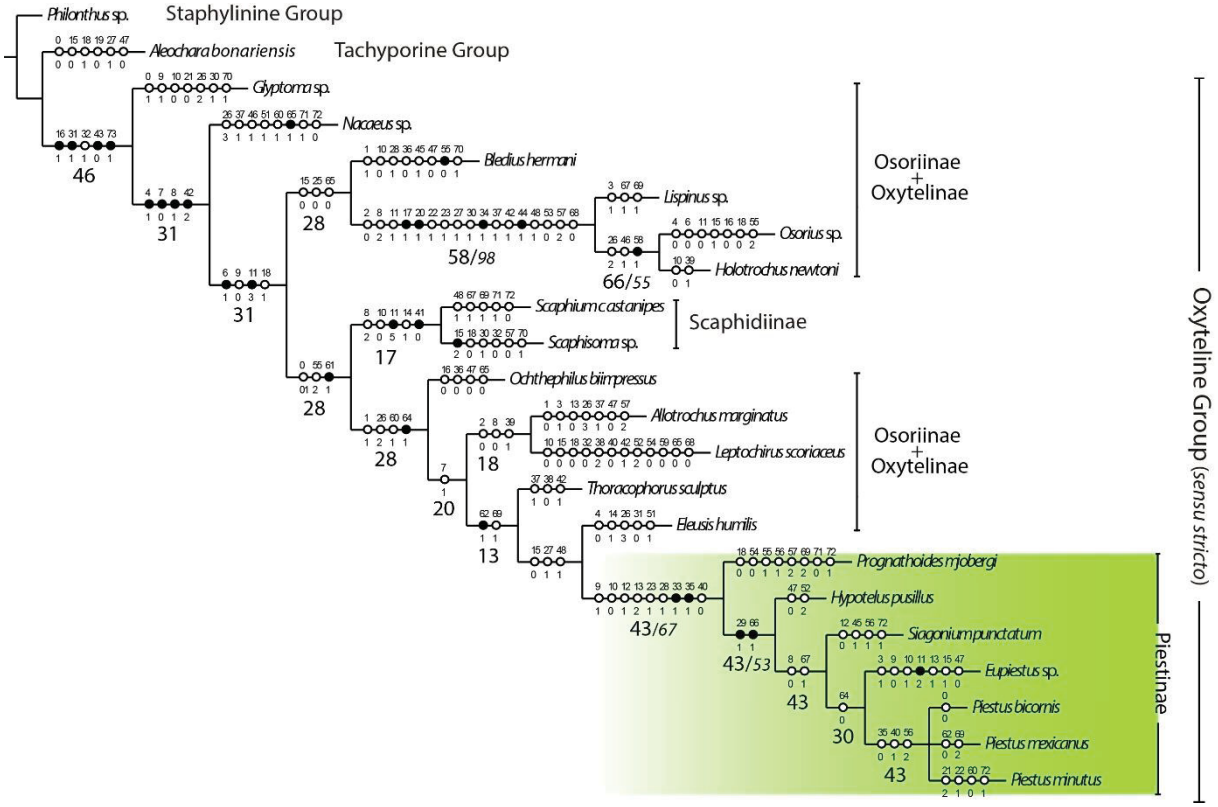

Figure 12. Reference topology (larval) – Implied weights analysis, strict consensus. Black circles indicate synapomorphic transformations and white circles indicate homoplastic transformations. The character number is shown above the circle and the corresponding character state is shown below it. The number displayed under each branch corresponds to the value of relative Bremer support and *Bootstrap*.

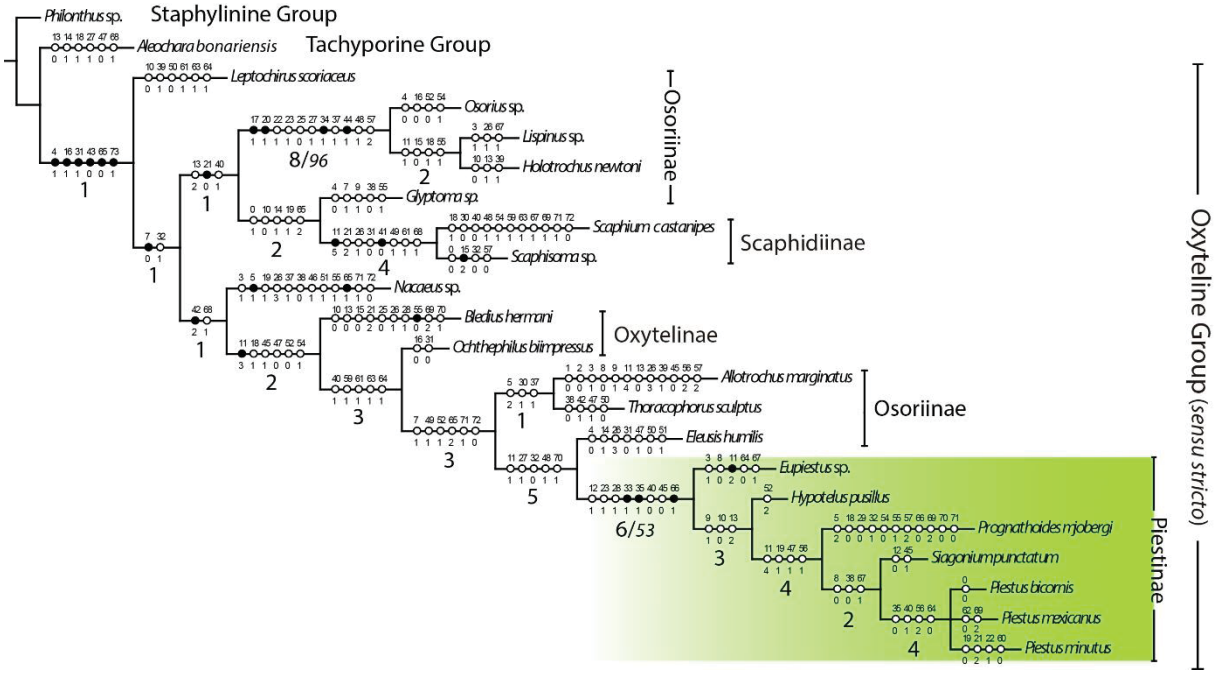

Figure 13. Equal weights analysis (larval) – strict consensus tree of two most parsimonious trees obtained. Black circles indicate synapomorphic transformations and white circles indicate homoplastic transformations. The character number is shown above the circle and the corresponding character state is shown below it. The number displayed under each branch corresponds to the value of absolute Bremer support and *Bootstrap*.

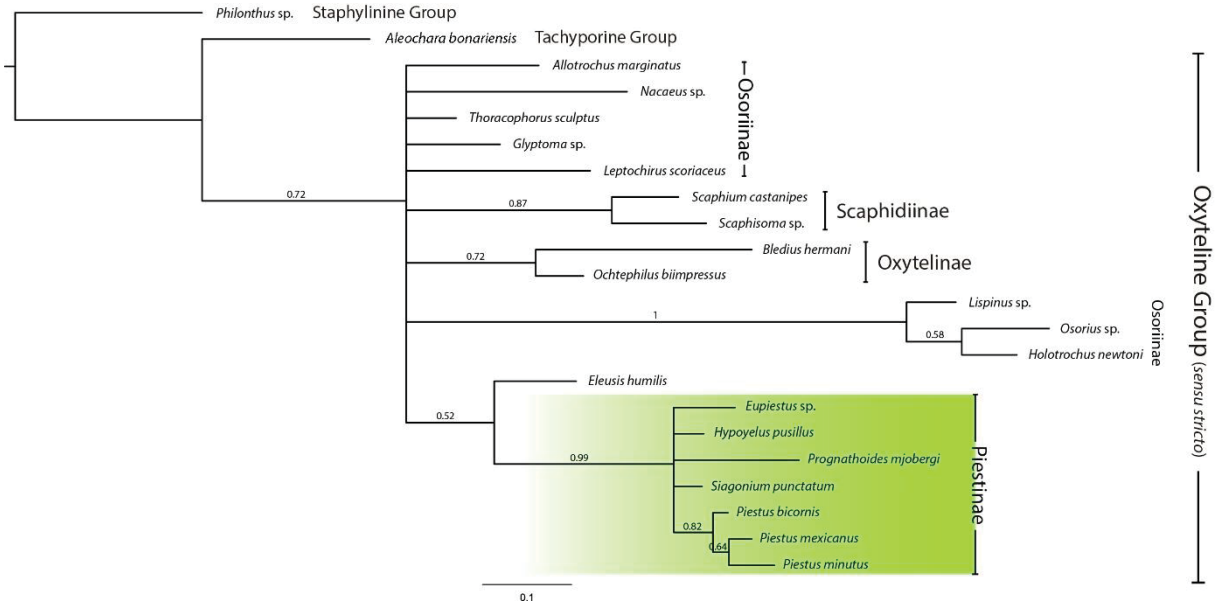

Figure 14. Majority rule (50%) consensus tree from the Bayesian analysis (larval). The number displayed above each branch corresponds to the value of the posterior probabilities.

5.3. DISCUSSION

5.3.1. Monophyly of the Piestinae lineage

As showed in Part I, here the results of the EW, IW and BI analyses recovered the monophyly of the subfamily Piestinae. Analyzing the results of Grebennikov & Newton (2012) (larval data only) is possible to note that neither Piestinae nor Oxyteline Group was recovered as a clade in all approaches carried out (Table 3, columns 1–4, in Grebennikov & Newton 2012). Beutel & Molenda (1997) showed a closer relationship of Piestinae, Osoriinae and Oxytelinae supported by having the mandibular apex with three or four strong apical teeth, though is not obvious to infer about the monophyly of Piestinae because they included only one terminal species of the subfamily in their study.

5.3.2. Larval versus adult morphological characters

Unlike that showed in Part I (adult morphological data), the larval approach could not resolve the relationship among the four subfamilies of the Oxyteline Group, only recovering as monophyletic clades the subfamilies Scaphidiinae and Piestinae. However, the values of the branch support for the clade Scaphidiinae is very low, indicating that the clade does not form a supported monophyletic clade. Furthermore, the topology recovered by Bayesian analysis shows that are only two branches well supported: 1) clade formed by the genera *Lispinus*, *Osorius* and *Holotrochus* (both Osoriinae), with PP= 1; and 2) clade formed by all larvae of Piestinae used in this work, forming a monophyletic group (PP= 0.99). This result indicates that these two clades were recovered by the analysis, and the others do not exist.

By the way, the larval data was not enough to providing a good phylogenetic signal with respect to internal relationship among piestines rove beetles, since we included only one terminal taxon per genus of Piestinae (excluding the genus *Piestus*), no apomorphic transformations (autapomorphic in this case) were achieved to support the clades, singly. Thus, the results of the analyses under the larval morphologic dataset are good to corroborate the results found in the adult morphologic dataset, in

recover the monophyly Piestinae but showed to be less effective to recover the monophyly of Piestinae members.

The results of the EW and IW and BI analyses suggest that *Eleusis humilis* is the sister-group of Piestinae. *Eleusis*, or members of the Tribe Eleusini, is clearly aberrant from other osoriines. Adults beetles have abdominal segments with a longitudinally membranous suture between terga and sterna instead of fused segments (Navarrete-Heredia *et al*. 2002). In addition, most adults are dorsoventrally depressed as an adaptation to their under-bark habitat, resemble species of the subfamily Piestinae (Irmler 2017).

The phylogenetic results of the analysis based on morphological evidences from adult suggested that *Eleusis* is a basal group in the osoriines clade, agreeing with A.F. Newton (personal communication), which, even if related closely to osoriines, it is probably at the base of the group. However, this hypothesis was not recovered with the approach based on morphology evidences in larvae, once the clade was not recovered as monophyletic.

5.3.3. Conflicts over larval morphologic characters used for taxonomic identification

Kasule (1968) characterized the subfamily Piestinae based on three different species larvae, *Siagonium quadricorne* and *Piestus* sp. (Piestinae) and *Nodynus leucofasciatus* (Apateticinae). At that time, he indicated some characters, which are not corroborated here, like the following:

- ocelli four or sometimes six (*Nodynus* – see Fig. 6 in Grebennikov & Newton 2012), arranged in a single oblique row. Four ocelli are present in *Piestus*, *Prognathoides* and *Siagonium*; *Hypotelus* have one ocellus, whereas *Eupiestus* are two. With respect to it arrange, they are arranged in a single oblique row, but it is in a curved oblique line instead of a straight one (Fig. 11H).
- Mandible with usually four apical teeth, a dilated molar-like base (Fig. 8 in Kasule 1968). The mandibles are usually four apically teethed, but they are asymmetrical. the right mandible has one less tooth.
- A single pleural plate on each side of the first 8 segments. Grebennikov & Newton (2012) scored for all piestines taxa used in their work as the absence

of laterotergites (=pleural plate) on abdominal segments I–VIII. Unfortunately, we did not score this character in the current work because most of the terminals have no laterotergites, but, after analyzing the larval piestines material, we agree with Grebennikov & Newton (2012).

- Spiracles placed in a membrane near the posterior lateral angles of tergites. This state of character was confirmed for most of larval, however, *Prognathoides* bear the spiracles in a region fully scloretinized of the terga.
- Urogomphi 2-segmented, with segment 1 much longer than segment 2. The genera *Hypotelus* and *Prognathoides* have a unique segment.

In addition, Hansen (1997) points out in the “Key to subfamilies of Staphylinidae (larvae)”, like characterization, that Piestinae larvae have the head with two large sclerites (mentum, submentum) between posterior tentorial pits, forming two distinct plates (Fig. 231 in Hansen 1997). After analyzed the larval piestines material, we have verified that all the Piestinae larvae used in this work have a distinct mentum and submentum, corroborating with Hansen (1997), although we did not code in our matrix.Thus, in the face of the material used in phylogenetic analysis (Table 5), we can affirm that this character corresponds to an apomorphic transformation to the subfamily.

Grebennikov & Newton (2012) did partially test this character. They described it at the character 58 as follows: “Mentum and submentum, ventral sclerotization [ventral view]: indistinct, if distinct, then transversely interrupted by membrane = 0”. Thus, the authors described it as a character bearing two states, “absent and present”, hidden the real information of the character.

6. PART III – MONOPHYLY OF PIESTINAE BASED ON MOLECULAR DATA

6.1. MATERIAL AND METHODS

In order to provide a complete source of evidence to support the hypothesis of the monophyly of the Subfamily Piestinae demonstrated in the previous parts (see items 4 and 5), we understand that an analysis based on molecular data would be necessary. The genes chosen to support the hypotheses presented in this part were: nuclear protein-coding: *wingless* – *Wg*; mitochondrial protein-encoding: Cytochrome Oxidase subunit 1 – *CO1*; and nuclear ribosomal: 28 rDNA – 28S. With the progress of the processes of extraction of the genetic material, PCR of the target sequences and their sequencing, we are faced with some problems that made it impossible to use the sequences extracted by us in this part. Thus, the results presented here come from data made available at GenBank.

6.1.1. Choice of terminals, genes and molecular methods

The choice of terminals and gene fragments were derived from previously published studies based on their performance at different taxonomic levels within Staphylinidae (Table 8), and, also, based on available gene sequences on GenBank.

Molecular data from three gene fragments (nuclear protein-coding: *wingless* – *Wg*; mitochondrial protein-encoding: Cytochrome Oxidase subunit 1 – *CO1*; and nuclear ribosomal: 28 rDNA – 28S) were used to create a concatenated alignment. Sequences alignments of each target gene were obtained in MFFT v.7 online version (https://mafft.cbrc.jp/alignment/server/) with default settings (algorithm: Auto; scoring matrix: 200 PAM/k = 2; gap open penalty: 1.53; and offset value: 0.123).

All three molecular markers (28S, CO1 and Wg) matrix data were performed, in most cases, for the same species. In cases where this was not possible because of available dataset limitations, different species were used. The choice was made based on species used in the same study or gene sequences obtained using the same primers from reference studies (28S – McKenna *et al*. (2015a); CO1 and Wg – Zhang & Zhou (2013)). Complete information might be understood by reading Table 8.

Table 8. List of specimens and gene sequences from GenBank selected in this study.

Subfamily	Species	28S	CO1	Wg
Staphylininae	*Philonthus* sp.	KJ844963.1 (*Philonthus caeruleipennis*) From McKenna *et al*. (2015a)	JX878801.1 (*Philonthus spinipes*) From Zhang & Zhou (2013)	JX878748.1 (*Philonthus spinipes*) From Zhang & Zhou (2013)
Aleocharinae	*Aleochara* sp.		JX878846.1 From Zhang & Zhou (2013)	JX878793.1 From Zhang & Zhou (2013)
Scaphidiinae	*Scaphium castanipes*	KJ845099.1 From McKenna *et al*. (2015a)	KU876137.1 unpublished	
	Scaphidium sp.	KP419633.1 From McKenna *et al*. (2015b)	JN299242.1 unpublished	KP813574.1 From McKenna *et al*. (2015b)
	Scaphisoma sp.	KJ845040.1 From McKenna *et al*. (2015a)	MG061944.1 unpublished	
Oxytelinae	*Bledius* sp.	KJ844972.1 From McKenna *et al*. (2015a)	JX878827.1 From Zhang & Zhou (2013)	JX878774.1 From Zhang & Zhou (2013)
	Oxytelus bengalensis		JX878832.1 From Zhang & Zhou (2013)	X878779.1 From Zhang & Zhou (2013)
	Homalotrichus substriatus	KJ845055.1 From McKenna *et al*. (2015a)		
	Oxypius peckorum	KJ844984.1 From McKenna *et al*. (2015a)		
	Carpelimus bilineatus	EF213821.1 unpublished	HQ953445.1 unpublished	
	Ochthephilus sp.	KJ845013.1 (*Ochthephilus planus*) From McKenna *et al*. (2015a)	JX878831.1 (*Ochthephilus emarginatus*) From Zhang & Zhou (2013)	JX878778.1 (*Ochthephilus emarginatus*) From Zhang & Zhou (2013)
Osoriinae	*Lispinus* sp.	KJ844907.1 (*Lispinus lineipennis*) From McKenna *et al*. (2015a)	JX878836.1 (*Lispinus quadricollis*) From Zhang & Zhou (2013)	JX878783.1 (*Lispinus quadricollis*) From Zhang & Zhou (2013)
	Nacaeus longulus		JX878837.1 From Zhang & Zhou (2013)	JX878784.1 From Zhang & Zhou (2013)
	Osorius freyi		JX878838.1 From Zhang & Zhou (2013)	JX878785.1 From Zhang & Zhou (2013)
	Eleusis sp.	KJ845091.1 From McKenna *et al*. (2015a)		
	Renardia sp.	KP419613.1 From McKenna *et al*. (2015b)		KP813558.1 From McKenna *et al*. (2015b)
	Plastus sp.	KJ844887.1 (*Plastus miles*) From McKenna *et al*. (2015a)	JX878841.1 (*Plastus unicolor*) From Zhang & Zhou (2013)	JX878788.1 (*Plastus unicolor*) From Zhang & Zhou (2013)
Piestinae	*Eupiestus* sp.	KJ845030.1 From McKenna *et al*. (2015a)		
	Piestus extimus	KJ844987.1 From McKenna *et al*. (2015a)		
	Siagonium americanum	KJ844988.1 From McKenna *et al*. (2015a)		

7.1.2. Phylogenetic analysis

The concatenated sequence alignment was analyzed under Bayesian analysis, carried out using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The following nucleotide substitution models were selected using jModelTest 2.1.10 (Darriba *et al*. 2012): GTR+I+G for both 28S and Wg; and GTR+G for CO1. Two Markov chain Monte Carlo (MCMC) runs of four chains were run for ten million generations.

The convergence was determined to have occurred when the standard deviation of split frequencies dropped below 0.005, also by the effective sample size (ESS) values higher than 200 in Tracer v.16 (Rambaut *et al*. 2014), as well as by the

examination of potential scale reduction factor (PSRF) values in the MrBayes output file.

The first 25% of trees were discarded as burn-in. The 50% consensus tree of the two MCMC runs was rooted with *Philonthus*. Branches with a posterior probability (PP)>0.95 were considered strongly supported; with PP=0.90–0.94 moderately supported; and with PP=0.85–0.89 weakly supported, as in Żyła *et al*. (2017).

6.2. RESULTS

6.2.1. Phylogenetic analysis

The concatenated molecular dataset comprised 2,391 aligned base pairs – 28S=1636pb; CO1= 844pb; Wg= 451pb. The Bayesian inference analysis (Fig. 15) converged after 10 million generations, with ASDSF= 0.0026 and nearly all PSRF values= 1.00 (minimum= 1.000 maximum= 1.015) and recovered broadly dissimilar phylogenetic patterns in relation to morphologic dataset (see items 4.2.2 and 5.2.2).

A major heterogeneous clade with Piestinae, Osoriinae, Oxytelinae and Scaphidiinae members was recovered (PP= 0.97), however the major of the subfamilies was found as paraphyletic. A strict relationship of Scaphidiinae is observed, but with no clade support (PP= 0.86). The tribes Eleusini (*Eleusis* + *Renardia*) and Thoracophorini (*Lispinus* + *Nacaeus*) were each recovered as monophyletic with strong support (PP= 1.00).

6.3. DISCUSSION

6.3.1. Non-monophyly of Piestinae

In contrast with the morphologic dataset of this work (see items 4.2.2 and 5.2.2), our molecular analysis Piestinae was demonstrated to be a non-monophyletic group as previous phylogenetic reconstructions have presented (Grebennikov & Newton

2012; McKenna *et al*. 2015). Historically, this subfamily has been suggested as a non-monophyletic group (Figs. 1–2) and poorly defined (see item 1.3).

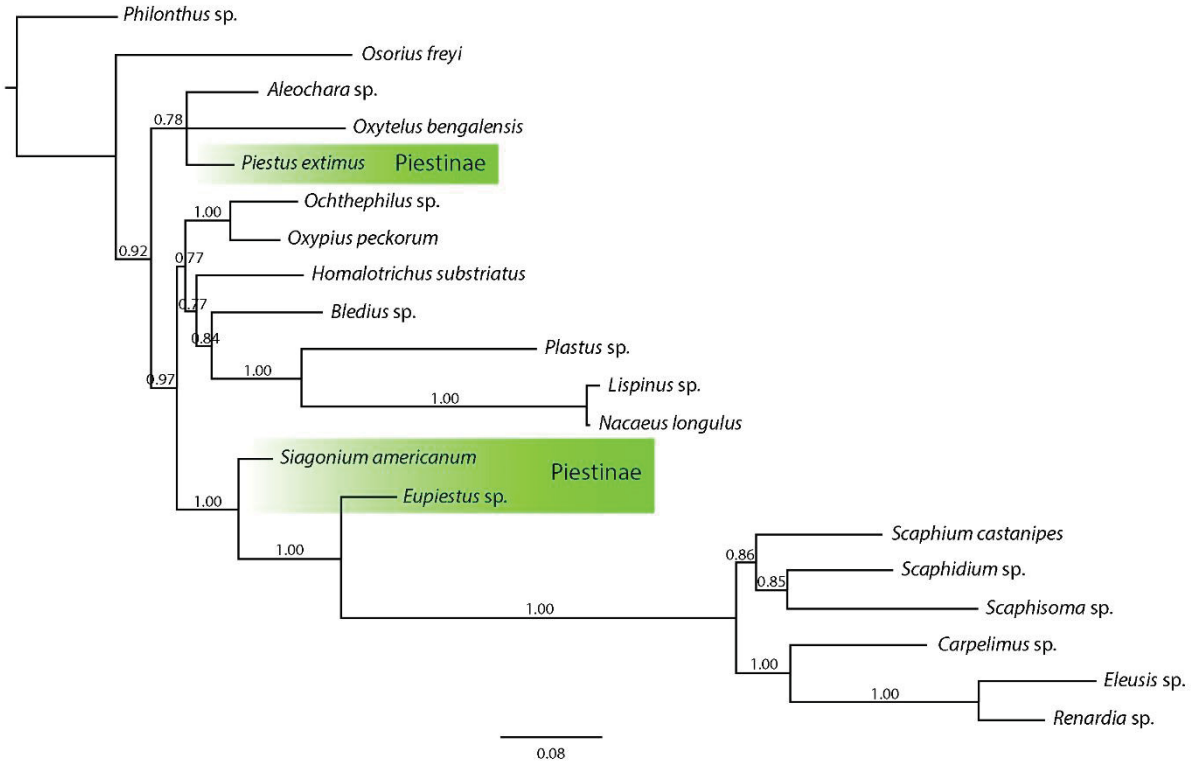

Figure 15. Majority rule (50%) consensus tree from the Bayesian analysis of three genes, with posterior probabilities above the corresponding branch.

McKenna *et al*. (2015) in both BI and ML (maximum likelihood) analysis demonstrated Piestinae to be paraphyletic/polyphyletic, respectively, with respect to the subfamilies Oxytelinae and Osoriinae combined. The authors included in their study 31 out of 35 subfamilies of Staphylinidae and used 2 nuclear genes (28S and CAD) and recovered the monophylum of 12 subfamilies, but neither Oxyteline Group nor Piestinae was recovered. We reached a similar result in our BI analysis, which might be partially explained because we used the 28S gene sequence from McKenna *et al*. (2015).

Zhang & Zhou (2013), using 28S, CO1 and Wg, analyzed 15 subfamilies and their relationships. Neither Oxytelinae nor Piestinae as well as any of subfamilies that compound the Oxyteline Group was recovered as monophyletic. Even Staphylininae, subfamily decisively monophyletic (Cai *et al*. 2019; Żyla & Solodovnikov 2019), was

found to be non-monophyletic. Part of their results seems to be caused by errors as point out by Gusarov (2018). Probably, the 28S gene sequence for oxyteline beetle *Ochthephilus emarginatus* does not correspond to the true sequence, also topology without branch length shown in the published trees making it more difficult to assess the causes of problematic groupings.

7. PART IV – MONOPHYLY OF PIESTINAE BASED ON MERGED MORPHOLOGICAL DATA

7.1. MATERIAL AND METHODS

In order to verify if the hypothesis of the monophyly of the Subfamily of Piestinae remain and its internal relationship, we performed analyzes using a matrix with the combined data sets of adults and larvae.

The combined larval and adult morphological matrix includes 37 terminals and all 238 characters (APPENDIX 9), resulting of merged matrices. The matrices were combined using Winclada with the command Matrix >> New matrix merged using the criterion Terminal match >> Match terminal by name. After we combined the adult and larvae matrices, a new terminal was added (*Eupiestus* sp.), because that terminal did not match by name. The missing characters were coded as not applicable '-' by the software.

Characters 1 – 164 refer to adult morphology; characters 165 – 238 refer to larval morphology. The matrix does not bear polymorphic character.

The phylogenetic analyses were carried out using the same parameters for adult and larval morphological data.

The TNT script (setk.run) written by Salvador Arias was used to calculate the appropriate value for the constant k (for details see Goloboff *et al*., 2008a). The script returned a value of k =5.781 for our data set, which was used. The choice to use this script here and not the one proposed by Mirande (2009) is because the script written by Mirande has read errors on some computers. The TNT software sometimes being able to read the script and sometimes not, as already explained by the author.

The recovered topologies by TNT were manipulated and edited in WinClada version 1.00.08 (Nixon 2002). Trees from the Bayesian analysis was visualized and edited in Figtree v1.4.2 (Rambaut 2014). Final editing was performed using Adobe Illustrator CS6 version 16.0.0. Only unambiguous character changes are shown on the trees.

7.2. RESULTS

The Maximum parsimony (MP) analyses under equal weights (EW) returned 39 most parsimonious trees with a length of 1052 steps, with CI= 0.28 and RI= 0.58. The topology of the strict consensus is shown in Fig. 17.

The analysis attributing IW generated 5 trees for the best *K* criterion (K value equal to 5.781) returned by the script. The best topology attributing IW generated 5 trees with 1053 steps and 73.495 adjusted fit and its consensus is shown in Fig. 16. RBS was calculated from 77,373 suboptimal trees and is shown below each clade branch.

The Bayesian inference analysis converged before 10 million generations and, at the end of the run, an average standard deviation of split frequencies had stabilized near to 0.005, while nearly all PSRF values were 1.000 (minimum = 1.000; maximum= 1.004). The majority rule consensus tree is shown in Fig. 18.

The topology of the strict consensus under EW anaysis recovered the same synapomorphic transformations for the clade of the Subfamily Piestinae presented in the topologies of adults and larvae (see item 4.2 for adults and item 5.2 for larvae) under IW analysis, but two synapomorphic transformations present in the topologies under EW analysis were not recovered in the topology under EW with the combined data set, they are: 152:2 = Tergum VIII, laterotergites distinctly demarcated from tergum VIII by suture (for adult morphologic dataset) and 66:1 = Urogomphi, length 1–1.5x compared to length of tergum VIII (for larval morphologic dataset). Besides of that, the topology under IW analysis recovered the same synapomorphic transformations presented in the other topologies under IW (Fig. 7 for adult and Fig. 12 for larvae) presented in this work.

The analyses still identified the monophyly of the Piestinae (ABS= 5; BT=91 for EW; RBS= 59; BT= 98 for IW; PP= 1 for BI), with two main clades: 1) *Piestus* + (*Hypotelus* + *Eupiestus*) and 2) *Siagonium* + *Piestoneus*. However, the analyses did not recover the same monophyletic clades. *Hypotelus* and *Eupiestus* were identified as not monophyletic with very low branch support (ABS= 1; BT= -- for EW; RBS= 33; BT= -- for IW; PP= 0.53 for BI). The genus *Siagonium* still was recovered as paraphyletic clade with respect to the genus *Piestoneus*, with branch support values for this clade: ABS= --; BT= -- for EW; RBS= 53; BT= 65 for IW; PP= -- for BI).

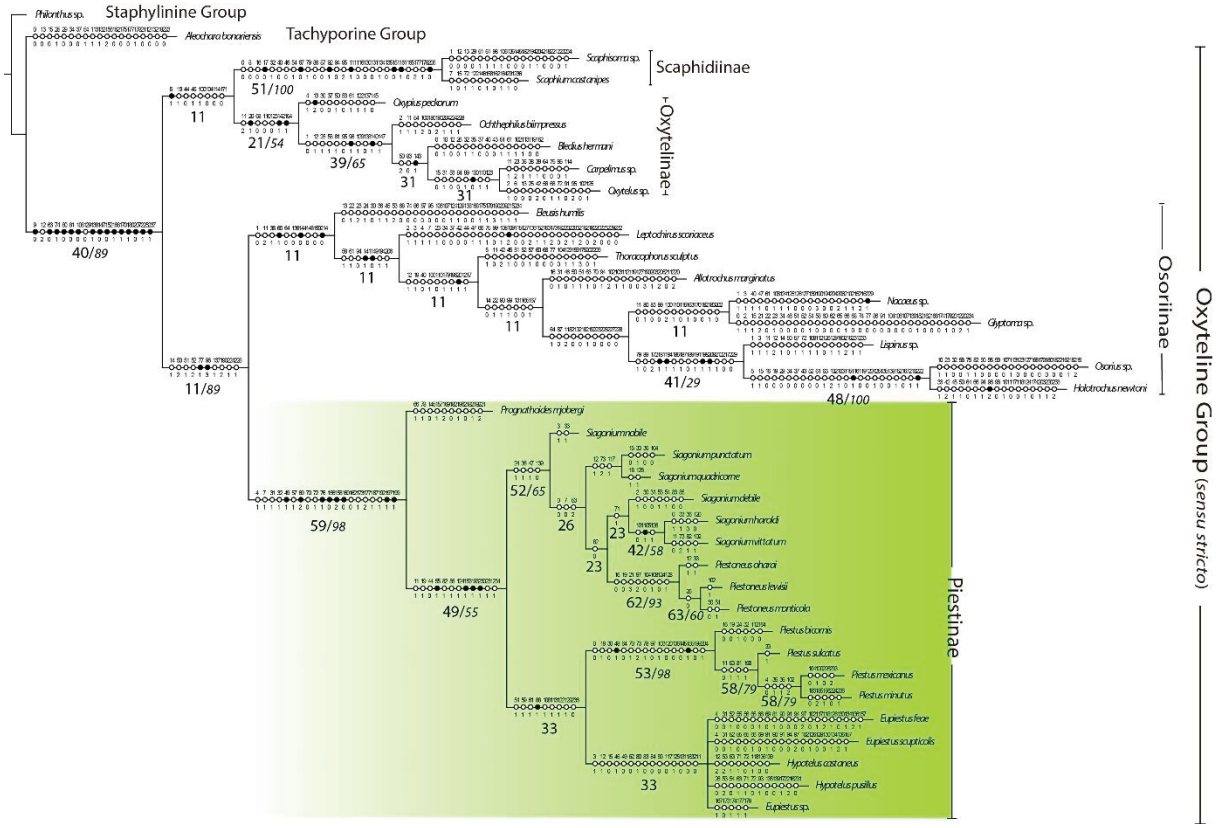

Figure 16. Implied weights analysis (combined) – strict consensus tree of five most parsimonious trees obtained: Length= 1079; CI= 0.27; RI= 0.57. Black circles indicate synapomorphic transformations and white circles indicate homoplastic transformations. The character number is shown above the circle and the corresponding character state is shown below it. The number displayed under each branch corresponds to the value of relative Bremer support and Bootstrap.

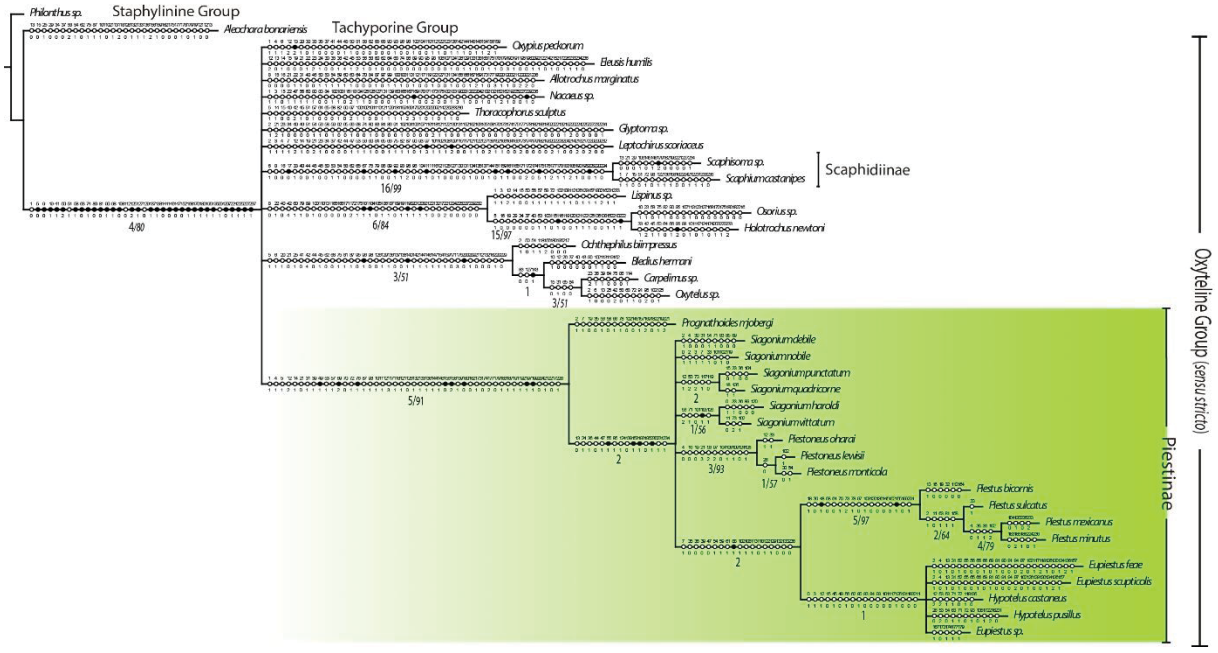

Figure 17. Equal weights analysis (combined) – strict consensus tree of 39 most parsimonious trees obtained: Length= 1239; CI= 0.23; RI= 0.48. Black circles indicate synapomorphic transformations and white circles indicate homoplastic transformations. The character number is shown above the circle and the corresponding character state is shown below it. The number displayed under each branch corresponds to the value of absolute Bremer support and Bootstrap.

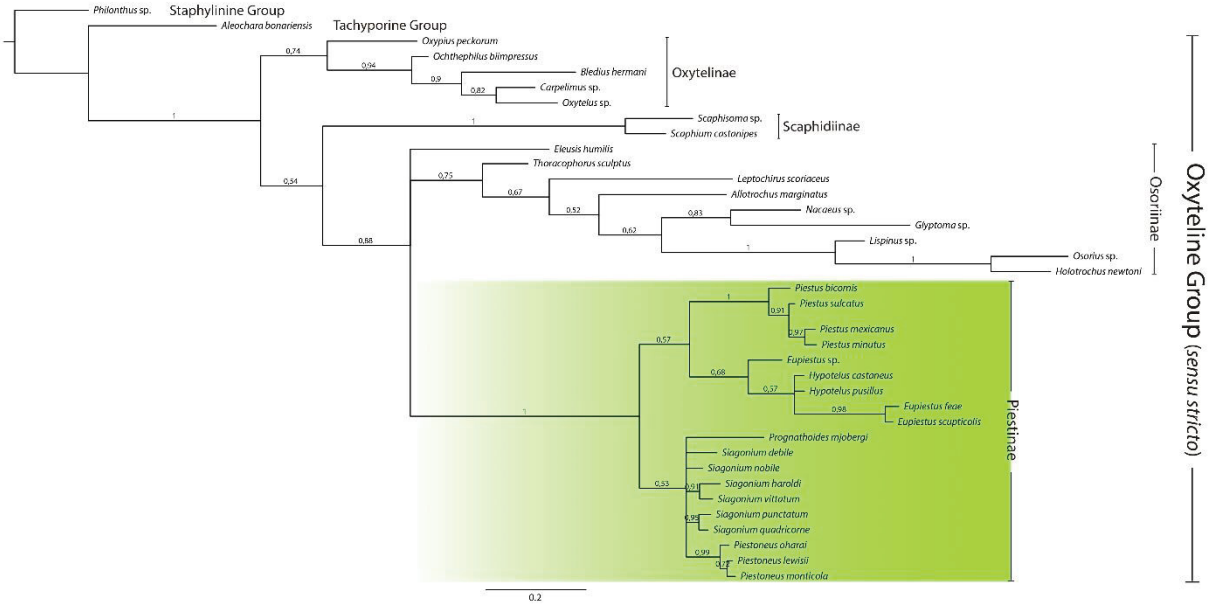

Figure 18. Majority rule (50%) consensus tree from the Bayesian analysis (combined). The number displayed above each branch corresponds to the value of the posterior probabilities.

7.3. DISCUSSION

As expected, the analyzes performed with the combined dataset recovered the subfamily Piestinae as a monophyletic clade. In all topologies, the same clade patterns, being these: *Piestus* + (*Hypotelus* + *Eupiestus*) and *Siagonium* + *Piestoneus*, were recovered compared to the analyzes performed with the independent datasets. A special mention should be made of the *Eupiestus* + *Hypotelus* clade in which the results indicate that these two genera may not be monophyletic groups, in contrast to the results obtained previously. We believe that this conflicting result is due to the terminal *Eupiestus* sp., added when we merged the two matrices into one, so, here, we discard these results and make the results presented in part I as valid (see 4.2.2). When analyzing the matrix, it is noticed that the characters referring to the adult portion for the terminal *Eupiestus* sp. were coded as not applicable, and this large amount of missing character may have influenced the resolution of the topology recovered by the analyzes presented in this chapter, as explained in Maddison (1993) and Kearney & Clark (2003).

With respect to the genus *Siagonium*, the analyses with combined datasets did not recover it as monophyletic, showing the same pattern of previous results. The absence of larval terminals of the genus *Piestoneus* may have hindered results more precise about the relations between theses genera. The combined results were also not satisfactory in determining the real positioning of the genus *Prognathoides* within the Subfamily. As shown in the results with separate datasets, *Prognathoides* occupies the most basal position within the Subfamily (analyses under parsimony) or might form a clade together with the genera *Siagonium* and *Piestoneus* (Bayesian Inference analysis).

Another important information that we may note when comparing the topologies between separate datasets and combined datasets concerns branch support. The monophyly of the Subfamily has remained, with strong support value in the IW and BI. However, but not surprising, in the analyzes with combined datasets the branch support values have decreased for some clades.

8. CONCLUSION

This study represents the first attempt to investigate the monophylum of the subfamily Piestinae based on three different dataset and here we presented dichotomic results. Piestinae was recovered as a monophyletic group under a morphologic dataset opposite to molecular dataset. However, in the face of the uncertainty of molecular sequences as mentioned earlier, we assume the monophylum instead of the non-monophylum of the subfamily.

Our study offers substantial progress in resolving the phylogenetic understanding of the subfamily Piestinae. However, in face to different hypotheses of the relationship obtained for the internal lineages, future work should focus on the addition of monotypic genus *Parasiagonum* to improve our understanding about of the systematics and evolution of *Siagonium* – once the present results demonstrated that this genus is not natural group – and the basis of Piestinae, coupled with genomic analysis.

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APPENDIX 1 – Label data and depository information for the adults used for scoring morphological characters for the phylogenetic analysis of the subfamily Piestinae (Coleoptera: Staphylinidae).

Staphylininae: *Philonthus* sp., Brazil, Paraná, Curitiba, Centro politécnico-UFPR, M.R. Silva col., DZUP. **Aleocharinae:** *Aleochara bonariensis*, Brazil, Paraná, Curitiba, Centro politécnico-UFPR, M.R. Silva col., DZUP. **Scaphidiinae:** *Scaphisoma* sp., No data, 685, MZUSP. *Scaphium castanipes*, Canada, Ontario, 89 mi. N. Pickle Lake, 21.VI.1973, Campbell & Parry col., FMNH. **Oxytelinae:** *Oxypius peckorum*, Australia, Western Australia, Beedelup National Park, 3.XII.1976, J. Kethly col., FMNH. *Bledius hermani*, Brazil, Paraná, Pontal do Paraná, 05.IV.2017, E. Caron col., DZUP. *Ochthephilus biimpresus*, USA, Oregon, Benton Co., Siuslaw N.F., Marys peak (NE side), Chintimini Ck., 16.V.2012, M. Thayer col., FMNH. *Carpelimus* sp. Brazil, Rio Grande do Sul, Rio Grande, Estuário Lagos dos Patos, VI.2012, K. Dummel col., DZUP. *Oxytelus* sp., Brazil, Pará, Uruará, VI.2015, Reinaldo, Wully & Idielson col., DZUP. **Osoriinae:** *Eleusis humilis*, Panama, Canal Zone, Barro Colorado Island, 10.II.1976, A. Newton col., FMNH. *Allotrochus marginatus*, Mexico, Veracruz, Canyon Rio Metlac near Fortin, 3200 ft., 28–31.VII.1973, A. Newton col., FMNH. *Lispinus* sp., Brazil, São Paulo, Peruíbe, 29.XI–01.XII.1984, Expedição MZUSP, col., MZUSP. *Nacaeus* sp., Brazil, São Paulo, Itanhaém, 09.IV.1980, Expedição MZUSP col., MZUSP. *Thoracophorus sculptus*, Australia, Victoria, Acheron Gap, 28–30.IV.1978, S. Peck col., FMNH. *Glyptoma* sp., Panama, Canal Zone, Barro Colorado Island, 25.II.1976, A. Newton col., FMNH. *Leptochirus scoriaceus*, Brazil, Rio de Janeiro, Nova Friburgo, 05–09.I.1981, Expedição MZUSP col., MZUSP. *Osorius* sp., Brazil, São Paulo, Alto da Serra, R. Splitz, col., MZUSP. *Holotrochus newtoni*, Mexico, Puebla, 5 mi. NE Tezi??tlán, 5000 ft, 17–19.VII.1973, A. Newton col., FMNH. São Paulo, Peruíbe, 29.XI–01.XII, Expedição MZUSP, col., MZUSP. **Piestinae:** *Eupiestus feae*, Tonkin, Thanh Moi, H. Perrot col., FMNH. *Eupiestus scupticolis,* India, Mysore 3mi. N. Yellapur 550m, 16.II.1962, E.S. Ross & D.Q. Cavagnaro col., FMNH. *Hypotelus cataneus*, Peru, Cuzco Dept., Consuelo, Manu rd., 11.X.1982. L.E. Watrous & G. Mazurek col., FMNH. *Hypotelus pusillus*, Brazil, Paraná, Palotina, UFPR, 12.XII.2016. Caron & Bortoluzzi col., DZUP. *Piestoneus lewisii*, Japan, Usui-Pass, Nagano Pref., Chubu-Distroit, 19.VI.1995, S. Nomura col., DZUP. *Piestoneus monticola*, Japan, Nishi-Tanzawa, Kanagawa Pref., Honshu, 27.VIII.1971, Y. Hirano col., DZUP.

Piestoneus oharai, Japan, Usui-Pass, Nagano Pref., Chubu-Distroit, 19.VI.1995, S. Nomura col., DZUP. *Piestus bicornis*, Brazil, Santa Catarina, Nova Teutonia, VII.1941, Dirings col., MZUSP. *Piestus mexicanus,* Mexico, Tamaulipas, Gómez Farias, 6.VI.1983, S. & J. Peck col., FMNH. *Piestus minutus*, Panama, Canal Zone, Madden Dan, 12.VI.1976, A. Newton col., FMNH. *Piestus sulcatus*, Brazil, Amazonas, Manaus, Reserva Ducke, 23.IX–04.XI.2014, T. Vicente col., DZUP. *Prognathoides mjobergi*, Australia, New South Wales, New England National Park, 30.XII.1990, Pollock & Reichert col., FMNH. *Siagonium debile*, Japan, Mt. Shinzan, Hakone, Kanagawa Pref., 21.V.1972, Y. Hirano col., DZUP. *Siagonium haroldi*, Japan, Mt. Tanzawa, Kanagawa Pref., Honshu, 26.VI.1983, Y. Hirano col., DZUP. *Siagonium nobile*, Japan, Nishi-Tanzawa, Kanagawa Pref., Honshu, 08.IX.1984, Y. Hirano col., DZUP. *Siagonium punctatum*, Mexico, Nuevo Leon, Cerro Potosi, 14–16.VII.1970, A. Newton, FMNH. *Siagonium quadricorne*, Austria, Österreich, Niederösterreich, Ulrichskirchen-Schleinbach, J. Spurny col., FMNH. *Siagonium vittatum*, Japan, Hokkaido, Hakodate, 13.V.1971, Y. Hirano col., DZUP.

APPENDIX 2 – Summary of tests carried out for the choice of the reference topology of adult morphological characters approach.

Trees used to construct the final hypothesis are in bold

K	dist	K (real value)	RI	IC	Steps	clad	fit	CD	RF	SPR
0	50,00%	2.682	61	26	740	1	75.504	0.9431	0.1922	0.8424
1	52,86%	3.007	61	26	734	1	71.911	0.9552	0.1471	0.9273
2	55,71%	3.374	62	26	734	1	68.247	0.9552	0.1471	0.9273
3	58,57%	3.792	62	26	734	1	64.538	0.9552	0.1471	0.9273
4	61,43%	4.271	62	26	734	1	60.779	0.9552	0.1471	0.9273
5	**64,29%**	**4.827**	**62**	**26**	**734**	**1**	**56.960**	**0.9552**	**0.1471**	**0.9273**
6	67,14%	5.480	62	26	734	1	53.076	0.9552	0.1471	0.9273
7	70,00%	6.258	62	26	734	1	49.118	0.9552	0.1471	0.9273
8	72,86%	7.199	62	27	733	1	45.064	0.9381	0.2059	0.8748
9	75,71%	8.361	62	27	733	1	40.914	0.9381	0.2059	0.8748
10	78,57%	9.833	62	27	733	1	36.664	0.9381	0.2059	0.8748
11	81,43%	11.759	62	27	730	1	32.292	0.9379	0.2020	0.8929
12	84,29%	14.384	62	27	730	1	27.790	0.9379	0.2020	0.8929
13	87,14%	18.177	62	27	729	1	23.144	0.9324	0.2078	0.8929
14	90,00%	24.136	62	27	729	1	18.342	0.9324	0.2078	0.8929

K – reference topology; dist – distortion; K – K value; IR – Retention Index; CI – Consistency Index; steps – number of steps; clad – numbers of cladograms; fit – adjust; CD – Distortion Coefficient average; RF – Robinson-Foulds distance average; SPR – SPR distance average. Tree used to construct the final hypothesis are in bold.

APPENDIX 3 – Data matrix of adult morphological characters used for the phylogenetic analysis of the subfamily Piestinae (Coleoptera: Staphylinidae).

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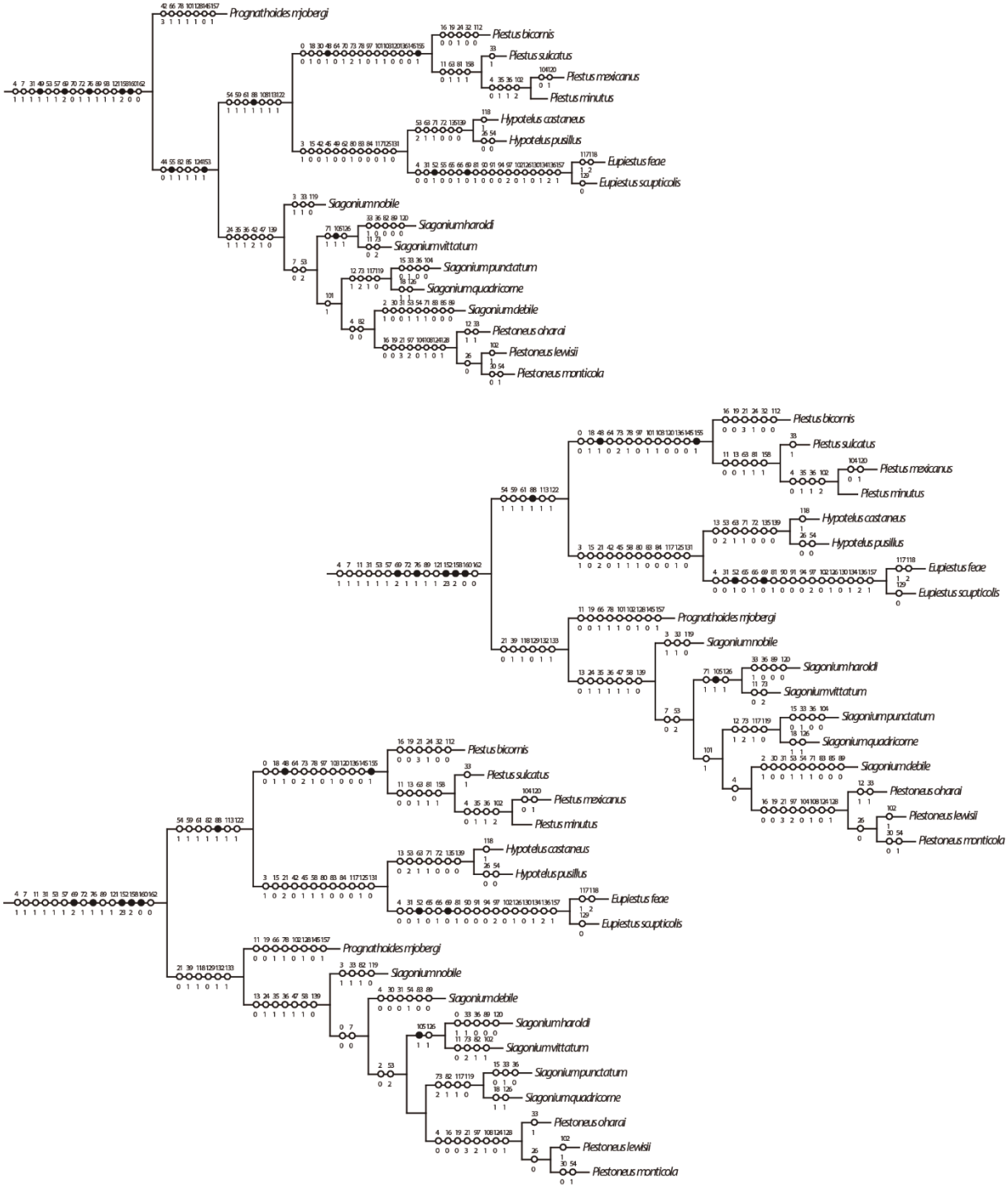
                          0    5    10   15   20   25   30   35   40   45   50 53   58   63   68   73   78
                          |    |    |    |    |    |    |    |    |    |    | |    |    |    |    |    |
Philonthus sp.            11100100010011011100010000011000011010010111010000000000000001111011110 0-000000110
Aleochara pseudochrysorroa 0100010011001000110001000111 10-0000--0-0103110100000021000000001110111100-010000110
Scaphisoma sp.            00000010000000001000100000011101011110100013101000010110000001001000111 00-110001000
Scaphium castanipes       01000011000021000001010000111110111101000121010000000110000000001000111010110001000
Oxypius peckorum          11001010100222011101110000111100011000-0101101100010000000000010010010---0-100000000
Bledius hermani           00000110101201011101100001011110101100-0004001100021210000100100111010---0-110000010
Ochthephilus biimpressus  10110110100111011101100001111110001001001041011000000110010000011010---0-110000010
Carpelimus sp.            10010110100111001101100201111111001011111041011000200000001000000010---0-100000010
Oxytelus sp.              10110100100210001101100000111111001001001021011000202000000000001001111011110000010
Eleusis humilis           10000000100220101100001210101100001011101141111000212200100010000101 0---0-000100000
Allotrochus marginatus    00000000000211000101001000111111001101100131100000112110001111110101110 00-100100000
Lispinus sp.              01010000000121101101011000101110101101000141001000012000111111101110111011100101000
Nacaeus sp.               01010000100011001101011000111110101101101111101100212000101010101101111 00-100100100
Thoracophorus sculptus    10000100100011101101010000101110101101101201111000200000010011000010---0-100000001
Glyptoma sp.              10100000100011011101020100101110100--11001111000002000101001010010110---0-000000100
Leptochirus scoriaceus    00111001100221111100020100101110000--0-0110100110021200101011100011111 00-110100001
Osorius sp.               00000100001211010100-1100111010000--0-0014000100021000000001011110111100-110101001
Holotrochus newtoni       00000100000211010100-1000111010110--0-0012011000110000001010110111111 00-100101000
Eupiestus feae            10110101100111101101020000100110101001001101011000201110??101001011100010101100111
Eupiestus scupticolis     11110101100111101101020000100110101001001101011000201110111101001011100010101100111
Hypotelus castaneus       10011101100120101101020000100111101001001101011000202211111101011101120 10-101100101
Hypotelus pusillus        11011101100110101101020000000111101001001101011000202201111101011101120 10-101100101
Piestoneus lewisii        01000100100120110100030010001111101111011121001101212201011000101101120010101100000
Piestoneus monticola      01000100100120110100030010001101101111011121001101212211011000101101120010101100000
Piestoneus oharai         01000100100110110100030010101111111111011121001101212201011000101101120010101100000
Piestus bicornis          00001101100121110110030010001101001001001141001011212111010101100101121012101110001
Piestus mexicanus         00100101100020111111010000100101101111001141001011202111 0??10111010112101210111001 1
Piestus minutus           00100101100020111111010000100101101111001141001011202111 01?10111010112101210111001 1
Piestus sulcatus          00101101100020111111010000000101111001001141001011202111 01010101110101121012101110011
Prognathoides mjobergi    11101101100021111100000000101111101001011131101001212100110000101111120010101110000
Siagonium debile          01100100100120111101000010101100101111011210011012121110110001011011201101011000000
Siagonium haroldi         11001100100120111101000010101111111101011210011012122010110001011011201101011000000
Siagonium nobile          11111101100120111101000010101111111111011210011012121010?10001011011200101011000001
Siagonium punctatum       01001100100110101101000010101111111101011210011012122010110001011011200121011000001
Siagonium quadricorne     01001100100110111110000101011111101111011210011012122010110001011011200121011000001
Siagonium vittatum        01001100100020111101000010101111101111011210011012122010110001011011201121011000001

                          83   88   93   98   103106  111  116  121  126  131  136  141  146  151  156159
                          |    |    |    |    | |   |    |    |    |    |    |    |    |    |    | |
Philonthus sp.            11100-0010010--012020-0211010101023010010001 0-1110000000-000-1111011110001000-10
Aleochara pseudochrysorroa 1101101010011 0-0021001021101011112121100100 00-1111101100-000-1111-111100020-11000
Scaphisoma sp.            11000-01111020-10---000110-11000-00???1010010-0000010001100 0-1010-100-000100 11110
Scaphium castanipes       11000-01111020-01110000210-11000-021001110010-0000010101100 0-110--100-000101 11100
Oxypius peckorum          -------0101110-02102000210-00100------0100211001100011111100101010-1110010200 11010
Bledius hermani           01011000100101-02101000110-00010-200010000200-1110001100-101111111010010100 11000
Ochthephilus biimpressus  11011001100101-022020-0210-000-0-2211100002011111000110 0-1010111101110010200 11010
Carpelimus sp.            00-01011100101-02002000210-10001023011001020 0-1110001100-101111111010010100 11010
Oxytelus sp.              00-11011000121-02000000110-100-0-2301100101010 0-1110001100-101111110101001010 0 11010
Eleusis humilis           11000-00100103102202010000-10001121011001120101110101010-000-0-0--1-10-00100 0--10
Allotrochus marginatus    10-11011100113011021102 10-00100-200010110210-0100011011101 0-0-0--0-10-00100 0--?0
Lispinus sp.              11110-01100013011101010211100101121211001020101100012111010-0-0--0-10-00100 ???10
Nacaeus sp.               00-10-11100013110201010211010001101211001110 0-0100001011001 0-0-0--0-10-00100 0--11
Thoracophorus sculptus    -------0100013212101000210-00000------1010211111100110111010-0-0--0-10-00100 11010
Glyptoma sp.              00-00-11000013110201000000-10000-012111102111000000101111010-0-0--0-11-00100 0--01
Leptochirus scoriaceus    11011010101033102110211210001020011111 0210-01100012111010-0-0--0-11-00100 0--11
Osorius sp.               11110-00100003-10102110200-000110112001100110-0100000210-010-0-0--0-11-00100 0--?0
Holotrochus newtoni       11010-01100112-01112110210-000010100001111111001000000 10-010-0-0--0-11-00100 0--00
Eupiestus feae            00-11110000013212200010211010111212111111111010001121110 0-1110010212000121 0000
Eupiestus scupticolis     00-11110000013212200010211010111120011111111100000011211 1000-1110010121000121 0000
Hypotelus castaneus       00-11111100113112201010211010111120111111110101100000111 0000-1110010121000021 0000
Hypotelus pusillus        00-11111101113112201010211010111120011111101010110000011 10000-1110010121000021 0000
Piestoneus lewisii        11111011101113212211000211110101123111101020110111101111 0000-1110010121000021 0000
Piestoneus monticola      11111011101113212212000211110101123111101020110111101111 0000-1110010121000021 0000
Piestoneus oharai         11111011101113212212000211110101123111101020110111101111 0000-1110010121000021 0000
Piestus bicornis          11111111101113011211110211010011230101111201011100010111 000-1010-1013101000 10000
Piestus mexicanus         11111111101113012212100211010111230111111201011100010111 000-1010-1013101001 10000
Piestus minutus           11111111101113012212110211010111230101111201011100010111 000-1010-1013101001 10000
Piestus sulcatus          11111111101113011211102110101111230101111201011100010111 000-1010-1013101001 10000
Prognathoides mjobergi    11011011101113112210010210-10101123111101020110111101111 0000-1010-1013000012 10000
Siagonium debile          01011001101113112212010210-10101123111101120100111101111 0000-1110010121000021 0000
Siagonium haroldi         11111001101113112202011210-10101123110101121100111101111 0000-1110010121000021 0000
Siagonium nobile          11111011101113112201010210-10101123101101120100111101111 0000-1110010121000021 0000
Siagonium punctatum       11111011101113112212000210-10101121101101120100111101111 0000-1110010121000021 0000
Siagonium quadricorne     11111011101113112212010210-10101121101101121100111101111 0000-1110010121000021 0000
Siagonium vittatum        11111011101113112201011210-10101123111101121100111101111 0000-1110010121000021 0000
```

APPENDIX 4 – Most parsimonious topologies of the Piestinae lineage's branch recovered by equal weights analysis using adult morphological characters.



APPENDIX 5 – Label data and depository information for the adults used for scoring morphological characters for the phylogenetic analysis of the subfamily Piestinae (Coleoptera: Staphylinidae).

Staphylininae: *Philonthus* sp., Brazil, Paraná, Curitiba, Centro politécnico-UFPR, M.R. Silva col., DZUP. **Aleocharinae:** *Aleochara bonariensis*, Brazil, Paraná, Curitiba, Centro politécnico-UFPR, M.R. Silva col., DZUP. **Scaphidiinae:** *Scaphisoma* sp., No data, 685, MZUSP. *Scaphium castanipes* Kirby, 1837, USA, New Hampshire, Coos Co., 0.7 mi S Jefferson Notch, 17.ix.1983, A. Newton & M. Thayer col., FMNH. **Oxytelinae:** *Bledius hermani*, Brazil, Paraná, Pontal do Paraná, 05.IV.2017, E. Caron col., DZUP. *Ochthephilus biimpresus*, USA, Oregon, Benton Co., Siuslaw N.F., Marys peak (NE side), Chintimini Ck., 16.V.2012, M. Thayer col., FMNH. **Osoriinae:** *Eleusis humilis*, Mexico, Veracruz, Balzapote, 07.VII.1976, A. Newton col., FMNH. *Allotrochus marginatus*, Mexico, Veracruz, Canyon Rio Metlac near Fortin, 3200 ft., 28–31.VII.1973, A. Newton col., FMNH. *Lispinus* sp., Brazil, São Paulo, Peruíbe, 29.XI–01.XII.1984, Expedição MZUSP, col., MZUSP. *Nacaeus* sp., Brazil, São Paulo, Itanhaém, 09.IV.1980, Expedição MZUSP col., MZUSP. *Thoracophorus sculptus*, Australia, Victoria, Mt. Margaret Rd. near Marysville, 17.ii.1993, A. Newton & M. Thayer col., FMNH. *Glyptoma* sp., Mexico, Chiapas, Ocozocoautla, 05.XI.1973, A. Newton col., FMNH. *Leptochirus scoriaceus*, Brazil, São Paulo, São Paulo, Parque Estação Cantareira, 03.III.1993, Expedição MZUSP col., MZUSP. *Osorius* sp., Brazil, São Paulo, Alto da Serra, R. Splitz, col., MZUSP. *Holotrochus newtoni*, Mexico, Puebla, 5 mi. NE Tezi??tlán, 5000 ft, 17–19.VII.1973, A. Newton col., FMNH. São Paulo, Peruíbe, 29.XI–01.XII, Expedição MZUSP, col., MZUSP. **Piestinae:** *Eupiestus* sp., Laos, Khammouan Pr., Ban Khoumkhan (Nahin-Nai), 4.vi.2008, A. Newton & M. Thayer col., FMNH; *Hypotelus pusillus*, Panama, Canal Zone, Barro Colorado Island, 08–25.II.1976, A. Newton col., FMNH. *Piestus bicornis*, Panama, Canal Zone, Madden Dam, 12.VI.1976, A. Newton col., FMNH. *Piestus mexicanus*, Mexico, Chiapas, Palenque, 100 m, 02–05.VII.1983, S & J Peck col., FMNH; Mexico, Veracruz, Balzapote, 07.VII.1976, A. Newton col., FMNH. *Piestus minutus*, Panama, Canal Zone, Madden Dam, 12.VII.1976, A. Newton col., FMNH. *Prognathoides mjobergi*, Australia, Queensland, Lamington N. P., 25.III.–04.IV.1985, J. & N. Lawrence col., FMNH. *Siagonium punctatum*, USA, Arizona, Pima Co., Mt. Lemmon, 5.IX.1974, J. Lawrence col., FMNH.

APPENDIX 6 – Summary of tests carried out for the choice of the reference topology of larval morphological characters approach.

Trees used to construct the final hypothesis are in bold.

K	Dist	K (real value)	IR	IC	Steps	clad	fit	CD	RF	SPR
0	50,00%	1.969	51	32	301	2	35.082	0.9483	0.1798	0.9474
1	52,86%	2.208	51	32	301	2	33.535	0.9483	0.1905	0.9509
2	55,71%	2.478	51	32	301	1	31.962	0.9449	0.1833	0.9474
3	58,57%	2.784	51	32	301	2	30.359	0.9483	0.1905	0.9509
4	61,43%	3.136	51	32	301	1	28.721	0.9475	0.1762	0.9474
5	**64,29%**	**3.545**	**51**	**32**	**300**	**2**	**27.044**	**0.9538**	**0.1705**	**0.9509**
6	67,14%	4.024	51	32	300	2	25.318	0.9538	0.1726	0.9474
7	70,00%	4.595	51	32	300	2	23.543	0.9541	0.1726	0.9509
8	72,86%	5.286	51	32	300	1	21.716	0.9537	0.1723	0.9474
9	75,71%	6.140	52	32	299	1	19.822	0.8993	0.2548	0.9439
10	78,57%	7.221	53	33	294	1	17.833	0.8602	0.4228	0.9474
11	81,43%	8.635	53	33	294	1	15.766	0.8602	0.4228	0.9439
12	84,29%	10.563	53	33	294	1	13.623	0.8602	0.4228	0.9439
13	87,14%	13.348	53	33	292	1	11.394	0.8296	0.5727	0.9474
14	90,00%	17.724	53	33	291	1	9.043	0.8130	0.5802	0.9474

K – reference topology; dist – distortion; K – K value; IR – Retention Index; CI – Consistency Index; steps – number of steps; clad – numbers of cladograms; fit – adjust; CD – Distortion Coefficient average; RF – Robinson-Foulds distance average; SPR – SPR distance average. Tree used to construct the final hypothesis are in bold.

APPENDIX 7 – Data matrix of larval morphological characters used for the phylogenetic analysis of the subfamily Piestinae (Coleoptera: Staphylinidae).

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                          0    5    10   15   20   25   30   35   40   45   5052   57   62   67   72
                          |    |    |    |    |    |    |    |    |    |  | |    |    |    |    |
Philonthus sp.            22--0-01--24010100010110-0000-0000--10200101000111100013200100-00201010110
Aleochara pseudochrysorrhoa 01--0-010210-01000100100-1110-0000001010011100000-02102020000-00200100010
Scaphium castanipes       10101010200512111011020 0-1100-001000101000---00111101112110101010201110101
Scaphisoma sp.            00101010200512121001020 0-1100-100000101010---00101101102100001000200101011
Eleusis humilis           11100-111011011010100100-1310-0000001010112001011101101201011111120011 1101
Allotrochus marginatus    10011211011400011010010 0-1300-1110001111112000000110111222011101120010 0101
Lispinus sp.              20011010201102001110101110110-1110101110111010011 0-02001020000-00001010011
Nacaeus sp.               201111001210-10110010100-1300-0110001100012000110 0-12101010010-00100100101
Thoracophorus sculptus    21101211101301011010010 0-1200-1110001100111001010100111201011111120011 0101
Glyptoma sp.              10--0-010100-21110010000-1200-1110001000111000010 0-01101210000-00200001011
Leptochirus scoriaceus    01001011020401001000010 0-1200-1100001021011000010100200221001101100000 0011
Osorius sp.               20000-002010-2010100101110210-1110101110111010111 0-00012021000-00000000011
Holotrochus newtoni       20001010200111001110101110210-1110101111111010111 0-01001121000-00000020011
Bledius hermani           21101010100310001010020 0-0101001100000100120010000-00110110000-00000121011
Ochthephilus biimpressus  01101010101301010010010 0-1200-0010000010112001000 0-00012110111011000100011
Eupiestus sp.             11111011001211011010010101211101010110100120000011101012010111110211111101
Hypotelus pusillus        11101011110112001010010101211101010110100120000011102012010111111210111101
Piestus bicornis          01101011010412001011010101211101010100011200001111010122101111102111111 01
Piestus mexicanus         11101011010412001011010101311101010100011200001111010122101110102111211 01
Piestus minutus           11101011010412001010021101311101010100011200001111010122101011102111111111
Prognathoides mjobergi    11101211110412001001010101211001110110100120000111101001120111111200120011
Siagonium punctatum       11101011010402001011010101211101010110000120010111101012110111111211111111
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APPENDIX 8 – Molecular dataset matrix and Bayesian parameters.

```
#NEXUS
begin data;
dimensions ntax=20 nchar=2931;
format datatype=dna interleave=yes gap=- missing=?;
matrix
Philonthus_sp.
------------------AGTAGCGGCGAGCGAACAGGGAAGAGCCCAGCACCGAATCCCGCGGCCGC-TCAGGTCGCCGGGAAAT
GTGGTGTTTGGGAGGGTCCGCCATCCCGAGGTCGCGCGGCGCGTCCAAGTCCTTCTTGAACGGGGCCACTTACCCACAGAGGGTGC
CAGGCCCGTTGCGATCGCCGCCGGCCGCCGGGAGGACCTCTCCTCAGAGTCGGGTTGCTTGATAGTGCAGCCCTAAGCGGGTGGTA
AACTCCATCTAAGGCTAAATATGATCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGG-GAGATTCAGCGTGTCTCTCGTCCGCC
CGCGA------------------------GCGTGCGAGCGTG-TCGACGG-TCGGCG----TACGCG-----------CCGGCCG
CGCC--------GCTCGCCGCCGCATACGGGTGGCGAACGCGTGCACTTCTCCCCCAGTAGGACGTCGCGACCCGTTGGGCGCGGG
CCT-AAGGACCGGCGGGG-CAGACCGCGCGG----------------------CGCCTTCACGGGCGTCCGCTCGGACCCCCGGC--
TCCCGGCCCGCTCGCTCGACGGTACGAC-------AGTGGCGC-GGGGCCGCG-------------------------ACTCGT-
------TCGCGTCCGGCCCGCCGCAAGTGCTCGCGGCA--AATCCGGCTGCCTCGGACCCAGTGCCGACACCGGACCCGTCGAGAC
TGCTGGCGGCGGTGT----CCTCGGACAGACCCCGTTTACACACGCCCGT-CGGCGACGCTATAGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTTATAGCGAAACCCACAGGCGCAATGAAAGTGAAGG
CGCGCCTTGCGCGCGC----CAAGGGCGGATGCGACG-GCC-----AACGT------------------GCCGACGCCGCACGCCC
GGGGCGTCTCGCGCTCATCGCGAGCTGAGGCGCACCCAGAGCGTACATGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCACCCGG--TATGAGCGAGTCTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGACCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTCGAACG
-ATGAAGCCGCGAG-ACACATATATAC-----ATATATACGGATCA-----------------------------------------
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GG
Aleochara_sp.
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Scaphium_castanipes
ACTAACTAGGATTCCCTTAGTAGCGGCGAGCGAACAGGGAAGAGCCCAGCACTGAATCCCGCGGCCGAAACCGGACGCCGGGAAAT
GTAGTGTTTGGGAGGGTCCGCCATCCCGAGGTTGCGTGGCGCGTCAAAGTCCTTCTTGAACGGGGCCACTTACCCATAGAGGGTGC
CAGGCCCGTAG-GACCGCCACCGATCG-CGGGAGGACCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCAAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATATGACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGATCGAATGGG-GAGATTCAGCGCGGCTCTTACTGGGC
GCCC-----------------------------GGTATCCGAG--TGACGC--CGGCC----TTCGGG------------TCGAGCG
CGC-------------------------CGGTCGACGAACGCGTGCACTTCTCCCCTAGTAGGACGTCGCGACTCGTTGGGTGTCGG
CCT-GAGG-ATTGCGGAG-GAGCCCGTGCGG----------------------TCTTG--------TGCCGCGCGGACCTGTGAC--
TCCCGGCCGACCGGCCCGACGATATAAAG------AATGGCGC-AGGGCCGCG-------------------------ACTTG--
------TCGCGTCTGACCCGTCGCAAGCACATACGGAACTAACCCGGTGG--TCGGACCTAGTGCCGATCCCGGACCCCTA-TGGC
TGTTAGCGGCGGTGT----TCTCAGACAGACCCTG-----GTAAGCCAGT-CTGCGACGCTTTAGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTA---ACGAAACCTAAAGGCGTAATGAAAGTGAAGG
CGCGCCTTGCGCGCGC----CGTGGGAAGATGGGCCG-AAC-----TTCGG-----------------TTTGGCCCCGCACTCCC
GGGGCGTCTCATTCTCGCAGAGAA--GAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGT--CTGGTGCGAGTTTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAAAT-
-GTGAAGCCGTGAGTATCGAATCAGAG-------------------------------------------------------------
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Scaphidium_sp.
------TAGGATTCCCCCAGTAGCGGCGAGCGAACAGGGAAGAGCCCAGCACCGAATCCCGCGGCCGACACCGGACGCCGGGAAAT
GTGGTGTTTGGGAGGGTCCGCCATCCCGAGGTCGTGCGGTGCGTCCAAGTCCTTCTTGAACGGGGCCACTTACCCATAGAGGGTGC
CAGGCCCGTAGCGACTGCCGCCGATCG-CGGGAGGACCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCAAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATATAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
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TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGTTCGAATGGG-TAGATTCAGCGCGTCTCCCGCCGGAC
GCTC-------------------------------GGTAACCGTT-GTGACGT--CGACC----TACGGG-----------TCG-GCG
CGCCGG---------TCCGACGATAC-CGGTGTTGGAACGCGTGCACTTCTACCCTAGTAGGACGTCGCGACCCGTTGGGTGTCGG
CCT-GAGG-CTCGTGGTG-GAGCCCGTGCGG-------------------CATACTC--------TGCCGTGCGGACCCGCGAC--
GCCCGACCGACAGGCCCGACGGTACGAC-------AATGGCGC-GGGGCCGCG--------------------------ACTTGT-
------TCGCGTCCGACCCGTCGCAAGCGCGGACGGTT--CACCCGACTG--CCGGACCTAGTGCCGACACCGGGCCCGTC-CGGC
TGCTAGCGGCGGTGC----TCTCGGACAGACTCCG---ATACACGCCGGT-CGGCGACGCTATAGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGATTA---GCAAAACCTAAAGGCGAAATGAAAGTGAAGG
CATGCCTTGCGCAGGC----CGAGGGAGGATGGGTCT-TGC-----TACGG-----------------CACGGACCCCGCACTCCC
GGGGCGTCTCGTTCTCATTGCGAGTTGAGGCGCACCTAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGT---GCGAGCGAGTTTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAACT-
-GTGAAGCCGCGAG-ACTTGATCGAATCAGAGTGCCAAGTGGGCCACTTTTG-----------------------------------
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10  Scaphisoma_sp.
ACTAACTAGGATTCCCTCAGTAGCGGCGAGCGAACAGGGAGGAGCCCAGCGCTGAATCCCGCGGCTTCA--CGGCCGCCGGGAAAT
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CAGGCCCGTAGCGACAGCCGCCGGCAG-CGGGAGGACCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCAAAGTGGGTGGTA
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TTCAACAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGTTCGAAAGGG-GAGATTCAGCGCGGCTCTGGCCGGCG
GGAT-----------------------------CGGGTCGCCGCGCGACGTCGCGTCC----TTCGGG-----------ACGCTTCG
CGCC--------GGCCCCGACCG----TCCGGTCGGAACGCGTGCACTTCTCCCCCAGTAGGACGTCGCGACCCGTAGGGCGTCGG
CTT-AAGG-AGCGAGGTG-GAGACCCGGCGA-------------------------------CTTCGGTCGCCGGGACCCGCGCC--
TCCGAGCCGACCTGCCCGACGGAATGACA------CATGGCGA-GGGGCCGCG--------------------------TCTCGC-
------ACGCGTCCGACCCGCCGCAAGCTCGAGTCGGC-CAACCCGTCGT--CCGGACCTAGTGCCGGCGGCGGGCCCGCT-CTGC
TGCTGGCGGCGGCGT----TCTTGGACAGACCTCTGG-TGACACGCCGGT-CAGCGACGCTATTGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTC---TATAAACCTAAAGGCGAAATGAAAGTGAAGG
CCCGCCTCGCGCGGGC----CGAGGGAGGATGGGTGC-GGC-----TTCGG-----------------CTGCGCCCCGCACTCCC
GGGGCGTCTCGTTCTCATTGCGAGTTGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCA----TTGCGAGTTTCATCTGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAAACG
-GTGAAGCCGCGAG-AATCGAACGAATCAGAG-------------------------------------------------------
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11  Bledius_sp.
ACTAACTAGGATTCCCTTAGTAGCGGCGAGCGAACAGGGAGAAGCCCAGCACCGAATCCCGCGGCCGACACCGGACGCCGGGAAAT
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CAGGCCCGTAGTGACCATCGCCGATTT-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATATAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGATCGAATGGA-AAGATTCAGCGCGTCTTCGGGTCGGC
GAGT-----------------------------CGTGCAGCTG--CGACGG-AAGTTGCGTTTACGTG-----------ACTTTCG
CGCT--------CTGTACGGCCGTGT-CCGGCAAGGAACGCGTGCACTTCTTTCCTAGTAGAACGTCGCGACCCGTTGGGTATCGG
TCT-AAGG-CCCGCGGTG-GAGGCCGTGTGT--GTGCGTTCG--------CGCGCAT--------TCACGCTCGGACCCGCGGAAT
GCCCGACCGATTCGCCCGACGGTTTGAAC------GAAGGCGC-TGGGCCGCG-----------------------ACTTGT-
------TCGCGTTTGGCCCGTCGCAAGCGCAGACGGCA---AGCCGGAGT--TCGGACCAAGTGCCGACTCTGGTCCCGTT-TCGC
TGCTGGCGGCGGTGC----TCTCAAACAGTGCTCA-----ACACGCCGGT-CAGCGACGCTATAGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTT---GCGAAACCTAAAGGCGAAATGAAAGTAAAGG
CGCGCCTTGCGCGCGC----CTAGGGAGGATGGGTCGTTAGCTTAATTAAG-----------------CGCGGCCCCGCACTCCC
GGGGCGTCTCGTTCTCATTGCGAGTTGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGATCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTAAGAATTGGGTATAGCGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCGTATTATGCGAGTCTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAA---
--TGAAGCCGCGAG-ATCGGATCAGAG-------------------------------------------------------------
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12  Carpelimus_sp.
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-------------------GGAGTCTAGCATGTGCGCGAGTCATTGGGACTT---AATAAACCTAAAGGCGAAATGAAAGTAAAAG
CACGTCTTGCACGTGG----TTAGGGAGGATGGGTTG-CAC-----TGCGG-----------------TGCGACTCCGCACTCCC
GGGGCGTCTCGTTCTCATTGCGAGTTGAGGCGCACCAAGAGCGTACATGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
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     ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
     TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGT---ATGTACGAGTCTCATCCGGTAA
     AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAAAGC
     -ATGAAGCCGCGAG-ATCGGATCAGAG-----TGCCAAGTGGGCCACTTTTGGTAAGCAGAACTGGCGCTGTGGGATGAACCAAAC
     GCCGAGTTAAAGCGCCAAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCATGGAAG
     TC
13   Ochthephilus_sp.
     ACTAACTAGGATTCCCTTAGTAGCGGCGAGCGAACAGGGAAAAGCCCAGCACCGAATCCCGCGGCCGTAACTGGACGCCGGGAAAT
     GTGGTGTTTGGGAGGGTCCGCTATCCCGAGATCGCGCGGCGCGTCCAAGTCCTTCTTGAACGGGGCCACTTACCCATAGAGGGTGC
     CAGGCCCGTAGTGACCGCCGTCGACCG-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGTGGGTGGTA
     AACTCCATCTAAGGCTAAATATGACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
     TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGATCGAATGGG-AAGATTCAGCGCGTTTCCGGGTCGGC
     GAGC----------------------------TGCGGTGTTC-GCGACGG-ACGTCG----TTCGCG------------GCGCCCG
     CGCT--------ATCCGTGGCCGTGT-CCGGCAAGGAACGCGTGCACTTCTTCCCTAGTAGGACGTCGCGACCCGTTGGGTGTCGG
     CCT-GAGG-CCCGCGGTG-GAGCCCGCGCGG--ACGGT-------------TCGCCG--------TTCCGCTCGGACCCGCGGTC-
     GCCCGGCCGACTCGCCCGACGGTTTGAC-------ATTGGCGC-TGGGCCGCG--------------------------ACTCGT-
     ------TCGCGTCCGGTCCGTCGCAAGTGCGGACGGTA---ACCTGGCAG--TCGGACCTAGTGCCGGCTCCGGGCCCGCC-CCGC
     TGCTGGCGGCGGAAC----CCTCGGACCGCGCCCTA--ACGCGCGCCGGT-CGGCGACGCTATAGCTTTGGGTACTTTCAGGACCC
     GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGATTT---GCTAAACCTAAAGGCGTAATGAAAGTGAAGG
     CGCGCCTTGCGCGCGC----CTAGGGAGGATGGGTCGTCGT-----TACGG-----------------CGCGGCCCCGCACTCCC
     GGGGCGTCTCGTTCTCATCGCGAGATGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
     ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
     TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGC--TTTGTGCGAGTCTCATCCGGTAA
     AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTGAACG
     CATGAAGCCGCGAG-ATCGGATCAGAG-----------------------------------------------------------
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14   Oxypius_peckorum
     ACTAACTAGGATTCCCTCAGTAGCGGCGAGCGAACAGGGAAGAGCCCAGCACCGAATCCCGCGGCCGACTTCGGACGCCGGGAAAT
     GTGGTGTTTGGGAGGGTCCGCCATCCCGTGATCGTGCGGCGCGTCCAAGTCCTTCTTGAACGGGGCCACTTACCCAGAGAGGGTGC
     CAGGCCCGTAGAGACCGGCGCCGATCG-CGGGAGGACCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGTGGGTGGTA
     AACTCCATCTAAGGCTAAATACGACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
     TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGATCGAATGGG-AAGATTCAGCGCGTTTCCGGGTCGGC
     GAGC----------------------------CGCGGTGTTC-GCGACGG-ACGTAGCGTTCGCGCT------------ACGTCCG
     CGCA--------CTCTGCGGCCGCGT-CCGGCAAGGAACGCGTGCACTTCTTCCCTAGTAGGACGTCGCGACCCGTTGGGTGTCGG
     CCT-AAGG-CCCGCGGTG-GAGCCCGCGCGG--ACGGT-------------TCGCCG--------TTCCGCTCGGACCCGCGGTC-
     GCCCGGCCGACTCGCCCGACGG-TGGAC-------ATTGGCGC-AGGGCCGCG--------------------------ACTCGT-
     ------TCGCGTCCGGCCCGTCGCAAGCGCGGACGGTACACACCTGGCAG--TCGGACCTAGCGCCGACTCCGGGCCCGTA-CGGC
     TGCTGGCGGCGGGAC----CCTCGGACCGTGCCCCG--ATACGCGCCGGT-CGGCGACGCTATAGCTTTGGGTACTTTCAGGACCC
     GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCAAGTCATTGGGACTA---GCGAAACCTAAAGGCGAAATGAAAGTGAAGG
     CGCGCTTCGCGCGCGC----CTAGGGAGGATGGGCCGTTGC-----CGCGG-----------------CGCGGCCCCGCACTCCC
     GGGGCGTCTCGTTCTCATCGCGAGATGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
     ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
     TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCG-TTTGCGCGAGTCTCATCCGGTAA
     AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAAATA
     -GTGAAGCCGCGAG-ATCGGATCAGAG-----------------------------------------------------------
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15   Oxytelus_bengalensis
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16   Homalotrichus_substriatus
     ACTAACTAGGATTTCCTTAGTAGCTGCGAGCGAACAGGGAAAAGCCCAGCACCGAATCCCGCGGCCGTAACCGGACGCCGGGAAAT
     GTGGTGTTTGGGAGGGTCCGCTATCCTGCGATC-TGCGATGCGTCCAAGTCCTTCTTGAACGGGGCCACTTACCCATAGAGGGTGC
     CAGGCCCGTAGGGACCTTCGCCGTTTG-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGTGGGTGGTA
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AACTCCATCTAAGGCTAAATATGACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGATCGAATGGG-GAGATTCAGCGCGTCTCCGGGTCGGC
GAGT----------------------------CATTTGTGCG-GTGACGG-GTGTCG----TTCGCG------------GCGCCCG
CACA--------CTCCGTGACCGAGT-CCGACAAGGAACGCGTGCACTTCTCCCCTAGTAGGACGTCGCGACCCGTTGGGTGTCGG
CCT-AAGG-CCCGTAGCG-GAGCCCGCGCGGA-ACGGT-------------TCGCCG--------TTTCGCGAGGACCTACGGTG-
TCCCGGCCGACTCGCCCGACGGTTTGAC-------GTTGGCGCTGGGACCGCG--------------------------ACTCGT-
------TCGCGTTCGGCCCATCGCAAGCGC-GACGGTA---ACCCGGCGG--TCGGACCTAGTGCCGACTCTGGGCCCGTG-C-GC
TGCTGGCGTCGGATC----TC---GACGATGCTCA----AATACGACGTCGTCGCGACGCT-TTGCTTTGGGTACTTTCAGGANNN
NNNNNNNNNNNNNNNNNNNNNNNNNNAGCATGTGCGCGAGTCATTGGGACTT---ACTAAACCTAAAGGCGAAATGAAAGTAAAGG
C-CGCCTAGTGCGTGC----CTAGGGAGGATGGGTCGTTGC-----CACGG------------------CGCGGCCCCGCACTCCC
GGGGCGTCTCGTTCTCATTGCGAGATGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
CAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCAAT-ATTATGCGAGTCTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTCGAAC-
-GTGAAGCCGCGAG-ATCGGATCAGAG-------------------------------------------------------------
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17  Eleusis_sp.
ACTAACTAGGATTTCCCTAGTAGCGGCGAGCGAACAGGGAAGAGCCCAGCACTGAATCCTCGGTCGG----CAACGGCCAGGAAAT
GTAGTGTTTGGGAGGGTCCGCTAGCCCGAGATCG-GAGACGAGTCCAAGTCC-TCTTGAAGGAGGCCATTTACCCATAGAGGGTGC
CAGGCCCGTAGTGACCGTCGACGATCC-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGAAAGTGCAGCCCTAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATATAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAAGAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGTTCGAATGGG-GAGATTCAATGTGTCCCCCATGCTGG
GCCA----------------------------TTGTGTCGTG--TGACGG--CGGCG----TTCGCG------------TCGTTCG
CGCC--------GGCATGGTGCTGCA-TCGGCTGGGTGCGCGTGCACTTCTCCCCTAGTAGGACGTCGCGACCCGTTGGGTGTCTG
CTTCACGG-CTTGCCAAC-GAGTCCGGTGCG-GACGGCGC-------TTCGGCGTCG-------TATCGTAGCGGAGTGGTGAC--
GCCCGGCAGACTTGCCCGACGGTATAAAT------TCTAATAC-GTAGCCGCA----------------------------------
---GTAATGCCTCCGGCCCGGCGCAAGCACGGGCGGTT---ACCTGGATG--CCGGACCTAGTGCCGACACCGGGGCCGTT-CGAC
TGTTGGCGACGTGTG----TCTCGGACCGTCTGCG---------TATAGT-TGGCGACGCTTTTGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGCTCG--AGCGAAACCTAAAGGCATAATGAAAGTAAAGC
CGCCTTGTGCG---GT----CGAGGGAGGATGGGTGG-CCG-----TACGT-----------------GGTCGCCCCGCACTCCC
GGGGTGTCTCATTCTCATTGCGAGAGGAGGCGCACCTAGAGCGTAAATGCTGGGACCCGAAAGATGGTGAACTATGCTTGGCCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTATGAGCTGAGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGTGCTCGT------TACGAGTCCCATCCGGTAA
AGCGAATGATTAGAGGTATTGGGGTCGAAACGATCTCAACCTATTCTCAAACTTTAAATGGGTGAGAGCTCCGGCTTGCTTAA---
-ATGAAGCCGCGAG-AATGGATCAGAG-------------------------------------------------------------
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18  Lispinus_sp.
ACTAACTAGGATTCCCTTAGTAGCGGCGAGCGAACAGGGAGGAGCCCAGCACTGAATCCTTGGTCGG----CAACGGCCAGGAAAT
GTAGTGTTAGGGAGGATCTCTTATCCCACAATTG-AAGATGCGTTCAAGTCC-TCTTGAACGGGGCCATTTACCCATAGAGGGTGC
CAGGCCCGTAGTGACCGTTGACAATTG-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGATAGTGCAGCCCTAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATATAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAAGAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGTTCGAATGGG-GAGATTCAGTGCGTCTCCTTTATATG
GACCGTGCGG----------------------TGTCTGTTTG--TGAAGG--CGGTG----GTCGCGTTTACGCGCGCTCCATTAG
CCTC--------ACCAGTCTCTCGGT-GCCATTGGGAGCGCGTGCACTTCTCCCCTAGTAGGACGTCACGACCCGTTGAGTGTCTG
TTT-ACGG-CTTACGGTTTGCGTCCGTTTTG--GCAGTTATGCACTTCACGGTGTTTAATTGTTTTCCATAATGGGGCTGTAAT--
GCCTAACAGACTCGCTCGACGGTATTATACGTTGCGAATGTAT-GCAGGCGCG----------------------------AGCTATT
TACGTAGCGCATTCGACCTATTGCAAGTATAAACGGTT---ACCCAGATG--CCGAACCTAGTTTCGACGCTGGGGCCGTT-TGGC
TGTTCGCAATAGTGC----TCTCGAATTGTCTGCA---------TACGGT-CTGTGACGCTATTGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTA-TAGCGAAACCTAAAGGCATAATGAAAGTAAAAT
CACTCTTCAAAGTGAT----TAAGGGAGGATGGGCTC-CTT-----TACTTTTGAAATACAGA-GTAAGAGGGGCTCCGCACTCCC
GGGGTGTCTCATTCTCATTGCGAGAGGAGGCGCACCTAGAGCGTACATGCTGGGACCCGAAAGATGGTGAACTATGCCTGGCCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTATGAGCTTGGTATAGGGGCGAAAGAC
TAATCGAATCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCAT------TACGAGTCCCATCCGGTAA
AGCGAATGATTAGAGGTATTGGGGTCGAAACGACCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAAG--
-CTGAAGCCGCGAG-AATGGATCAGAG-------------------------------------------------------------
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19  Nacaeus_longulus
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Osorius_freyi
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Plastus_sp.
ACTAACTAGGATTCCCTGAGTAGCGGCGAGCGAATAGGGATGAGCCCAGCACTGAATCCGAGGTCGG----CGACGGCCAGGAAAT
GTAGTGTTAGGGAGGATCCGCTATCCTGCGAT-ATTAGGTGTGTCAAAGTCA-ACTTGAACGGGGCCACATACCCACAGCGGGTGC
CAGGCCCGTAGCGACCGCTGAGTATCG-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGATAGTGCAGCCCTAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATATAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAAGAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGTTCGAATGGG-GAGATTCAGTGTGTTTCGGAGCGTGG
GGGCGTGTCGTGGGGTAAAACCACGCACATTGTGCGTCCGAG--TGACGG--CGGCG--TCCTTGTGGC---------GTCGGACG
CGCCGGCACGGTGCGCCCTGCAGAAC-CTCGCATCGAACGCGTGCACTTCTCCCCTAGTAGGACGTCGCGACCCGTTGGGTGTCCG
CCT-AAGG-CCCGTGGTT-CAGTCCGTCATCGGCCCGTTC-------TTCGGAGCGGG----TTTTCGATGTCGGATCCGCGGTC-
GCCCGGCAGACTGGCCCGACGGTATGTTGTGTATTTTATGTAC-GGGACCGCACATGCATACCATCATCTCGGTGGGGTGTGTGT-
------GTGCGTCTTGCACGTCGGCAAGTGCGTTCGTT---CTCGAACTG--TCGGACCTGGTGCCGATATTTGGGGCGTT-CGTC
TGTTGGCTGCTGTGTGATATTTTGTGTGGAGTCAGAC-GTTCCGTACAGT-CTGCGACGCTAATGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTACGCGAGTCATTGGGACTT--TGCGAAACCTAAAGGCGAAATGAAAGTGAAGT
GCCGCATCTTGTATGCGGTACGAGGGAGGATGGGCGGGTGGTCGTGTGTGTCTCGTGCATACACGTCCCACCTACCTCGCACTCCC
GGGGTGTCTCGTTCTCATTGCGAGATGAGGCGCACCTAGAGCGTCCATGCTGGGACCCGAAAGATGGTGAACTATGCCTGGGCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTATGATCTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCAT------TGCGAGTCCCATCCGGTAA
AGCGAATGATTAGAGGTATTGGGGTCGAAACGACTTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAA---
-CTGAAGCCGCGAGAATCGGATCAGGG----------------------------------------------------------
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Renardia_sp.
------TAGGATTTCCTTAGTAGCGGCGAGCGAACAGGAAAGAGCCCAGCACTGAATCCTCGGTCGG----CAACGGCCAGGAAAT
GTAGTGTTTGGGAGGGTCCGCTAGCCCGAGGTGA-TAGACGAGTCCAAGTCC-TCTTGAACGGGGCCATTTACCCATAGAGGGTGC
CAGGCCCGTAGAGACCGTCGACAATCT-CGGGAGGACCTCTCCTTAGAGTCGGGTTGCTTGAAAGTGCAGCCCTAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATACAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAAGAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGTTCGAATGGT-GAGATTCAGTGCGTCACCTGGCTGGG
CCGC-----------------------------TGTTACCGTG--TGACGG--GGGCG----CTCGCG------------CCATTCG
CACC--------GGTGCGGTGTTGCA-TCAGTTGGGTACGCGTGCACTTCTCACCTAGTAGGACGTCACGACCCGTTGGGTGTCTG
CCT-AAGG-CTTGTCAAC-GAGTCCGTTGCG-GGCGGTGC-------TTCGGCATTG-------TTTTGCTGCGGAGTGGCGAT--
GCCCGGCAGACTTGCCCGACGGTATGA--------ATTTGTAT-GTGGCCGCA-------------------------ATTTAT-
-------TGCATTCGGCCCGCGGCAAGCACAGTCGGTT---ACCCGGCTG--CCGGACCTAGTGCCGACACTGGGGCCGTC-TGAC
TGTTGGCTGCGTGTG----TCTCGAACAGACCATG---------TACAGT-CGGTGACGCTATTGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTC-TAGCGAAACCTAAAGGCGTAATGAAAGTAAAGC
CGACTTGTGCG---GT----TTAGGGAGGATGGGTGA-TCG-----TTCGC------------------GGTCGCCCCGCACTCCC
GGGGTGTCTCATTCTCATTGCGAGAGGAGGCGCACCTAGAGCGTATATGCTGGTACCCGAAAGATGGTGAACTATGCTTGGCCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTATGAGCTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCAT------TACGAGTCCCATCCGGTAA
AGCGAATGATTAGAGGTATTGGGGTCGAAACGACTTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTAA---
-CTGAAGCCGCGAGAATTGGATCAGGG-----TGCCCAGTGGGCCACTTTTG--------------------------------
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Eupiestus_sp.
ACTAACTAGGATTCCCTCAGTAGCGGCGAGCGAACAGGGAAAAGCCCAGCACCGAATCCCGCGGTC---TCAGGCCGCCGGGAAAT
GTGGTGTTAGGGAGGGTCCGCTTTCCCGAGGTCGTGCGGTGCGTCCAAGTCCTTCTTGAACGGGGCCACTTACCCACAGAGGGTGC
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CAGGCCCGTAGCGACCGCCGCCGATCG-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGCGGGTGGTA
AACTCCATCTAAGGCTAAATATGACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAACGGGCGAGATTCAGCGCGTCTCCGGTTTCGC
GAGT----------------------------CGCGGAGCTG--CGACGT-GCGTCG----TCCGCG------------ACGCGCG
CGCT-------CTCCCGCGGCCGCGT-TCCGC---GAACGCGTGCACTTCTCCCCTAGTAGGACGTCGCGACCCGTCGGGTGTCGG
CCT-AAGG-CCCGCGGTG-GAGCCCGCGCGG--ACGGT-------------TCGCCG------TTTTCCGCGCGGACCCGCGGCG-
ACCCGGCCGACCCGCTCGACGGTTTGAC-------ATTGGCGC-AGGGCCGCG------------------------ACACNCGT-
------TCGCGTCCGGCCCGTCGCAAGTGCGCGCGGTTCTCACCCGATCG--TCGGACCCGGTGCCGACCTCGGGCCCGCG-CTGC
TGCTGGCGGCGGAGT----CCTCGGACAGACCCTG----TACGCGCCGGT-CGGCGACGCTTTCGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACAC---TATAAACCTAAAGGCGCAATGAAAGTGAAGG
C-CGCCTCGCGCGTGC----CGAGGGAGGATGGGTCG-CGT-----CTCGG------------------CGCGGCCCCGCACTCCC
GGGGCGTCTCGTTCTCATCGCGAGTTGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGT--CGTGTGCGAGTCTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTGAACG
CTTGAAGCCGCGAG-ATCGGATCAGAG----------------------------------------------------------
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24  Piestus_extimus
ACTAACTAGGATTCCCCCAGTAGCTGCGAGCGAACAGGGAAGAGCCCAGCACCGAATCCCGCGGCCGTAACCGGACGCCGGGAAAT
GTGGTGTTAGGGAGGGTCCGCCATCCCGATGTCGCGCGTCGCGTCCAAGTCCTTCTTGAACGGGGCCACTTACCCATAGAGGGTGC
CAGGCCCGTAGCGACCGACGCCGGCGG-CGGGAGGACCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGTGGGTGGTA
AACTCCATCTAAGGCTAAATATAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAACGGG-GAGATTCAGCGCGTCTCCAGGTCGGC
GAGC----------------------------CGCGTCGTTCTGCGACAC-GTGACG----TACGCG------------TCGCGTG
CGCG--------ACACGCGGCCGAGT-CCGGCAGGGAACGCGTGCACTTCTCCCCCAGTAGGACGTCGCGACCCGTTGGGTGCAGG
CCT-ACGG-CCCGCGGTG-GAGCCCGTTCGG--ACGGCTTCG-----------GCCG--------CTTCCGTCGGACCCGCGTG--
GCCCGGCCTGCTCGCCCGACGGTCTGACA------AATGGCGT-GGTGCCGCG--------------------------AATTGT-
------TCGCGTCCGGCCCTCCGCAAGTGCGGACGGTA---ACCCGGCGG-CTCGGACCTAGCGCCGACCCCGGGCCCGTA-CGAC
TGCTGGCGGCGGTGT----CCTCGGACAGAGCACG---TTGCACGCCGGT-CGGCGACGCTATAGCTTTGGGTACTTTCAGGACCC
GTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGAATT---AATAAACCTAAAGGCGAAATGAAAGTGAAGG
CGCGCCTTGCGCGCGC----CTAGGGAGGATGGGCCTACGC--CTACAGTG------------------CACGGCCCCGCACTCCC
GGGGCGTCTCGTTCTCATTGCGAGATGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGT--CTGGTACGAGTCTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTGAACG
CGTGAAGCCGCGAG-ATCGGATCAGAG----------------------------------------------------------
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25  Siagonium_americanum
ACTAACTAGGATTCCCCTAGTAGCGGCGAGCGAACAGGGAAAAGCCCAGCACCGAATCCCGCGGCCGAAACCGGACGCCGGGAAAT
GTGGTGTTAGGGAGGGTCCGCCATCCCGAGGTCGTGCGGTGCGTCCAAGTCCTTCTTGAACGGGGCCASTTACCCAGAGAGGGTGC
CAGGCCCGTAGCGACCGCCGCCGATCG-CGGGAGGATCTCTCCTCAGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGCGGGTGGTA
AACTCCATCTAAGGCTAAATATAACCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAG
TTCAATAGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGATCGAACGGG-GAGATTCAGCGCGTCTTCGGGTCGGC
GAGC----------------------------GGTGGAGTTT--CGACGG-GTGGCG--TTCGCGCG------------TCGCTCG
CGTG--------CTCCGCCGCCGAGT-CCGGCAAGGAACGCGTGCACTTCTCCTYTAGTAGGACGTCGCGACCCGTTGGGTGTCGG
CCT-AAGG-CCCGCGGAG-GAGCCCGTGCGG--ACGGCGGCGG---TTTCGCCGTCG--------TCCCGCTCGGACCCGCGGAA-
TCCCGGCCGACCTGCTCGACGG-TTGAC-------GTTGGCGT-GGGGCCGCG--------------------------ACTAGT-
------TCGCGTCCGGCCCGTCGCAAGTTCGGGCGGTT---ACCCGGCGG--TCGGACCTGGTGCCGACTCCGGAGCCGCA-CGGC
TGTTAGCGGCGGTGT----CCTCGGACAGACCCTG----CACACGCCGGT-CGGCGACGCTTTAGCTTTGGGTACTTTCAGGANNN
NNNNNNNNNNNNNNNNNNNNNNNNNNAGCATGTGCGCGAGTCATTGGGACTA---GCGAAACCTAAAGGCGTAATGAAAGTGAAGG
C-CGCCTCGCGCGTGC----CGAGGGAGGATGGGTCGTCGC-----CACGG------------------CGCGGCCCCGCACTCCC
GGGGCGTCTCGTTCTCATCGCGAGATGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGC---CTGCGCGAGTCTCATCCGGTAA
AGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGATCTCCGGCTTGCTTGAACG
CGTGAAGCCGCGAG-ATCGAATCAGAG----------------------------------------------------------
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26
27  Philonthus_sp.
--------ATTTTAATTCTTCCAGGATTCGGTATAATTTCCCACATTATCAGACAAGAAAGAGGTAAAAAGGAAGCATTTGGTACT
CTAGGAATAATCTATGCAATAATAGCAAT---TGGATTATTAGGATTTATTGTATGAGCTCATCATATATTTACTGTAGGAATAGA
TGTTGATACTCGAGCCTACTTCACTTCTGCAACAATAATTATTGCCATCCCCACAGGAATCAAAATTTTCAGTTGATTAGCTACAC
TTCATGGAAC------TCAAATTAATTATTCCCCTTCAATACTATGAGCTATCGGATTTGTATTTTTATT---------TACTGTA
GGAGGCCTAACTGGAGTAATTCTAGCTAACT------CATCAATCGACATTATTCTCCATGATACATATTACGTAG--TAGCCCAC
TTCCATTATGTATTATCAATAGGAGCAGTATTTGCTATCATAGCCGGAGTAGTTCAATGATTCCCTATTTTCACAGGTTTAACCCT
AAATGAGAAATTCCTTAAAATTCAATTTACAACAATATTTATTGGAGTAAATTTAACCTTTTTCCCCCAACACTTTCTTGGACTGG
CTGGAATACCTCGACGATATTCTGACTACCCCGATGCATACACACCTTGAAATGTCATCTCTTCTCTAGGGTCATTAGTTTCTCTT
TCTAGAATTTTTCTTCTTCTATTTACAATTTGAGAAGGTTTCATTTCATACCGTAAAAGAATCTCACCATTAAACTTACCTACATC
TATTGAATGACTACAACTTATGCCACCTGCTGAACATAGATAC----------------------------
28  Aleochara_sp.
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      ------CTAATTTTATTCTTCCAGGATTTGGAATAATTTCTCATATTATTAGACAAGAAAGAGGAAAAAAGGAAACTTTTGGAACT
      TTAGGAATAATTTACGCAATAATAGCTAT---TGGACTATTAGGATTTGTCGTTTGAGCTCATCATATATTTACTGTAGGTATAGA
      TGTAGATACTCGAGCATATTTTACATCTGCAACAATAATTATTGCTGTCCCTACAGGAATTAAAATTTTTAGATGATTAGCTACAC
      TTCACGGAAC------TCAAATTAATTACTCTCCTTCAATACTATGAGCATTAGGATTTGTATTTTTATT---------TACAGTT
      GGAGGATTAACAGGAGTAATTTTAGCTAATT------CATCAATTGATATTATTTTACATGATACATATTATGTTG--TTGCTCAT
      TTCCATTATGTATTATCAATAGGAGCAGTATTTGCAATTATAGCTGGATTTATTCAATGATATCCTTTATTTACAGGTTTAACATT
      AAATAATAAATTGTTAAAAATTCAATTTTTAACAATATTTATTGGTGTTAACATTACTTTTTTTCCTCAACATTTTCTAGGTTTAG
      CAGGAATACCTCGACGATATTCAGATTATCCTGATGCCTATACAACTTGAAATATAATTTCCTCAATTGGAAGATTAATTTCAATA
      GTAAGAATTATTTTTTTTATTTTTATTATTTGAGAAAGATTTATTTCAATACGAAAAAGAATTATTTCATTAAATTTATCATCATC
      TATTGAATGATTTCAATCAATACCTCCAGCTGAACACAGATATTCTGAATTACCTATATTAACTAACTTC
29    Scaphium_castanipes
      ----------------------------------------------------------TGAGCTGGAATAGTTGGTACTTCTCTTAGT
      CTAT---TAATTCGAGCAGAATTAGGAACCCCTGGTAGATTAATCGGTGATGATCAAATTTATAATGTAATTGTAACCG---CCCA
      TGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTGTAATTGGAGGATTTGGAAATTGAATAGTACCCTTAATACTAGGAGCTC
      CTGATATAGCATTCCCCCGAATGAATAATATAAGATTTTGATTACTGCCCCCATCATTATCATTACTTTTAATAAGAAGATTAGTA
      GAAAGGGGAGCAGGAACAGGTTGAACAGTTTACCCGCCCCTATCTTCTAACATCGCTCATGGAGGAGCATCCGTCGACTTAGCTAT
      TTTTAGATTACATT-----TAGCTGGGATTTCATCTAATTTAGGGGCTGTAAATTTCATTTCTACAATTATTAACATACGATCCAT
      TGGAATATCATTTGATCGAATACCTTTATTTGTTTTATCCGTCGCTATCACCGCCCTTTTACTACTATTATCTCTCCCCGTACTAG
      CAGGAG--CTATTACTATACTACTAACTGACCGAAATTTAAATACAGCATTTTTTGACCCTGCAGGAGGAGGAGACCCAATTTTAT
      ACCAACATTTATTT----------------------------------------------------------------------
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30    Scaphidium_sp.
      --------AACATTATACTT-----------------------TATTTTTGGTGCCTGAGCAGGAATAGTCGGGACATCTCTTAGA
      CTTC---TAATTCGTGCAGAACTTGGAACACAAGGAAGATTAATTGGTGATGACCAAATTTATAATGTTATTGTAACTG---CCCA
      TGCATTTGTCATAATTTTTTTTATAGTTATACCTATTGTAATTGGGGGATTTGGAAATTGATTAGTCCCCTTAATGCTAGGAGCCC
      CAGATATAGCATTCCCTCGAATAAACAATATAAGATTTTGACTATTACCCCCTTCACTATCTTTACTTTTAATGAGAAGACTAGTT
      GAAAGAGGGGCTGGAACAGGATGAACTGTTTACCCCCCCTTGTCCTCGAATATTGCCCATGGAGGAGCTTCAGTAGATTTAGCAAT
      TTTTAGACTACATT-----TAGCAGGAATTTCTTCAATTTTAGGGGCTGTAAATTTTATTTCAACGATTATTAATATACGAACAAT
      TGGGATATCATTTGATCAAATACCCCTATTTATCTGATCTGTCGGTATCACAGCGTTACTTCTCCTTTTATCACTCCCAGTATTAG
      CCGGAG--CAATTACTATACTTTTAACAGATCGAAACCTAAATACAGCATTTTTTGATCCAGCTGGAGGCGGTGACCCAATTCTTT
      ACCAACATTTATTT----------------------------------------------------------------------
      ----------------------------------------------------------------------
31    Scaphisoma_sp.
      --------TACTTTATACTT-----------------------TATCTTTGGGGCTTGATCAGGAATAGTAGGTACTTCACTAAGT
      CTTT---TAATTCGAGCTGAATTAGGGATACCTGGAAGATTAATTGGTGATGACCAAATTTATAATGTAATTGTTACTG---CCCA
      TGCATTTGTAATAATTTTTTTTATAGTTATACCTATTGTAATTGGAGGTTTCGGAAATTGACTAATTCCATTAATACTTGGAGCCC
      CTGATATAGCATTCCCCCGAATAAATAATATAAGATTTTGACTTCTACCCCCGTCATTGTCATTACTATTATTAAGTGCTATAGTT
      GAAAGAGGAGCTGGTACTGGATGAACAGTTTATCCCCCCTTATCTGCCAATATCGCTCATGGTGGAGCTTCAGTTGATTTGGCCAT
      TTTTAGTCTTCATT-----TAGCAGGAATTTCTTCAATTTTAGGAGCTGTTAATTTTATTTCAACCATTATTAATATACGAGCCTC
      AGGAATAAAATTTGATAGAATACCACTTTTTATTTGATCTGTAGCAATTACTGCTCTTTTACTTCTTTTATCACTACCAGTATTAG
      CTGGTG--CTATCACCATACTCCTAACTGATCGAAATCTTAACACAACCTTCTTTGACCCAGCAGGAGGAGGAGATCCAATTCTTT
      ACCAACATTTATTT----------------------------------------------------------------------
      ----------------------------------------------------------------------
32    Bledius_sp.
      ---------ATTTTATTCTTCCTGGATTTGGTATAATTTCCCACATTATTAGACAAGAAAGAGGAAAAAAAGAAGCTTTTGGAACT
      TTAGGAATAATCTATGCAATAATAGCTAT---TGGATTATTAGGCTTTATTGTTTGAGCCCATCATATATTTACAGTAGGAATAGA
      TGTTGATACTCGAGCTTATTTTACTTCAGCTACTATAATTATTGCAGTACCTACAGGTATTAAAATTTTCAGATGACTTGCAACTC
      TTCATGGAAC------TCAAATTAACTTTTCACCTTCTATACTTTGAGCATTAGGATTTGTTTTTCTTTT---------TACTGTT
      GGAGGATTAACAGGTGTTGTCCTAGCTAATT------CTTCAATTGATATTATCCTTCATGATACTTATTATGTTG--TTGCTCAT
      TTTCACTATGTTTTATCTATAGGTGCTGTATTTGCAATTATAGCTGGTCTAGTTCATTGATTTCCTTTGTTTACTGGAGTAACTTT
      AAACAATAAACTATTAAAAATTCAATTTTTTTCTATATTTATTGGAGTAAATATTACATTTTTCCCTCAACATTTTCTTGGATTAG
      CAGGCATACCTCGTCGATATTCAGATTACCCTGATGCTTATTTACCTTGAAATTTAATTTCCTCTATTGGTTCAATTATTTCTATA
      ATTAGAATTTTATTTATACTTTTTATTATTTGAGAAGGCTTTATTTCTATACGAAAAACTATCTCTCCTTTAAATTTATCTACTTC
      TATTGAATGAATACAACTTTTACCCCCAGCTGAACATAGTTATTCAGAACTTCCTATATTAACTAATTTC
33    Carpelimus_sp.
      --------AACTTTATATTT-----------------------TATTTTTGGGGCTTGATCAGGAATAGTAGGAACTTCCCTCAGA
      ATAC---TAATTCGGATTGAACTAGGAACCCCGGGATCATTAATTGGTGATGATCAAATTTATAATGTTATTGTTACGG---CCCA
      TGCTTTTATCATAATTTTTTTTATAGTAATACCAATTGTAATTGGTGGATTTGGAAACTGATTAGTCCCTCTAATATTAGGTGCCC
      CAGATATAGCATTTCCTCGAATAAATAATATAAGATTTTGACTTTTACCTCCCTCTTTAACCCTCCTATTATTCAGAAGAATAGTA
      GAAAGAGGGGCAGGTACAGGTTGAACTGTTTATCCCCCATTATCTTCTAATATTGCTCATAGAGGATCATCTGTTGATTTAGCAAT
      TTTTAGTCTTCATC-----TTGCAGGTATTTCATCAATTTTAGGGGCAGTAAATTTTATTTCAACAATTATTAATATACGTTCAAT
      TGGAATATCATTTGATCGAATACCATTATTTGTATGATCAGTTAATATTACAGCTATTCTTTTATTACTATCTTTACCTGTTTTAG
      CAGGTG--CAATTACTATACTATTAACAGATCGAAATTTAAATACATCATTTTTTGATCCTGCAGGAGGTGGCGATCCAATTTTAT
      ATCAACATCTATTT----------------------------------------------------------------------
      ----------------------------------------------------------------------
34    Ochthephilus_sp.
      ---------ATTTTATTTTACCAGGATTTGGAATAATTTCTCATATTATTAGCCAAGAAAGAGGTAAAAAGGAAGCTTTCGGAACC
      TTAGGAATAATTTATGCAATAATAGCAAT---TGGATTATTAGGATTTATTGTTTGAGCCCATCATATATTTACCATTGGAATAGA
      TGTTGATACCCGAGCTTATTTTACTTCAGCTACAATAATTATTGCAGTTCCAACTGGAATTAAAATTTTCAGATGACTTGCAACTC
      TTCATGGAAC------TCAAATTAATTATTCGCCCTCAATATTATGAGCCCTAGGATTTGTATTTTTATT---------TACAGTA
      GGAGGATTAACAGGTGTTGTATTAGCAAACT------CCTCAATTGATATTATTTTACATGATACTTATTATGTAG--TAGCACAT
      TTCCACTACGTCTTATCAATAGGAGCAGTATTTGCAATTATAGCAGGTTTAATTCAATGATTCCCATTATTTACAGGATTAACAAT
      AAATAATAAATTTTTAAAAATTCAATTTTTTTCTATATTTATTGGTGTAAATTTAACCTTTTTCCCCCAACATTTTCTAGGATTAG
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    CAGGAATACCCCGACGATACTCAGATTATCCCGATGCATATACCCCTTGAAATTTAATTTCCTCCATTGGTTCAATTATTTCTATA
    ATTAGTATTTTATTTATACTTTTTATTATTTGAGAAGCTTTTACTTCTATACGACAAAGATTATCCTCATTAAATTTTACATCTTC
    AATTGAATGAATACAACTTATACCACCTGCTGAACATAGATATTCTGAATTACCTATACTAACCCATTTC
35  Oxypius_peckorum
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36  Oxytelus_bengalensis
    -CCTGGGCTCTTTTATTTTACCAGGATTTGGTCTAATTTCCCATATTATTAGACAAGAAAGAGGTAAAAAAGAAGCATTCGGAACT
    CTTGGAATAATTTATGCTATATTAGCAAT---TGGATTATTAGGTTTCATTGTATGAGCTCACCATATATTTACCATTGGTATAGA
    TGTAGACACTCGAGCATATTTTACTTCAGCAACAATAATTATTGCTGTTCCAACAGGAATCAAAATTTTTAGGTGATTAGCTACTC
    TTCATGGAAC------ACAAATTAACTATTCCCCATCCATACTTTGAGCTTTAGGATTTGTTTTCTTATT---------CACTGTT
    GGAGGATTAACAGGAGTTATTTTAGCAAACT------CATCAATTGATATTGTACTTCATGATACATATTATGTAG--TAGCCCAC
    TTCCATTATGTTTTATCAATAGGAGCTGTATTTGCTATCATAGCTGGATTAGTCCATTGATTTCCTTTATTTACTGGTCTAACTTT
    AAATAATAATTTACTAAAAATCCAATTCTTTTGTATATTTGTAGGTGTAAATATTACATTTTTCCCACAACATTTCTTAGGATTGG
    CTGGAATACCACGACGATACTCTGACTATCCTGATGCTTATACCCCATGAAATCTTGTTTCTAGAATTGGGTCAATTATATCAATA
    ATTAGAATTTTATTTTTTTTAACTATTATCTGAGAAGGATTATCATCTAATCGAAAAAGACTTTCATCCTTAAATATAAATTCATC
    TATTGAATGAATACAATTAATACCTCCAGCAGAACATAGTTATTCCGAACTACCAATATTAATCAAATAG
37  Homalotrichus_substriatus
    ??????????????????????????????????????????????????????????????????????????????????????????????????
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38  Eleusis_sp.
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39  Lispinus_sp.
    CGGAACTAAATTCTATCTTACCCGGGTTTGGTTTAATTTCTCATATTATCAGCCAGGAAAGTGGGAAAAAGGAAACCTTTGGATCT
    CTAGGAATAATTTATGCTATATTAGCTAT---TGGATTCCTGGGATTTATTGTTTGGGCTCACCATATATTCACAGTAGGCATAGA
    CGTAGATACCCGAGCTTATTTTACATCAGCCACAATAATTATTGCAGTTCCGACCGGAATTAAAATTTTTAGATGACTAGCCACAC
    TTCATGGCAC------ACAAATTAATTATTCGCCGTCTATCCTCTGAGCTCTAGGTTTTGTATTTTTATT---------TACAGTA
    GGTGGACTAACAGGAGTAATTCTAGCCAATT------CTTCCATTGATATTATCTTACACGATACTTATTACGTAG--TAGCTCAT
    TTTCATTATGTTTTATCAATAGGAGCTGTTTTCGCTATTATAAGAGGTTTCATTCACTGATTTCCCCTATTTACTGGGCTCTCTCT
    CAGGGAAAAAATATTAAAAATTCAATTCATAATTATATTTATTGGAGTAAATTTAACATTCTTTCCCCAACATTTTCTGGGGCTTG
    CAGGTATACCACGACGATACTCAGATTACCCCGACGCATACACCCCTTGAAATGTAATTTCCTCGATTGGGTCTATTATATCTATA
    ATTGGCATTTTATTTATAATTTTTATCATCTGAGAAGCTTTTTCTGTCCAACGAAAGAGGATTTCCACTCTTAATTTTTCATCCTC
    AATTGAATGAAACCACTACATACCCCCGTCTGAACACACTTATTCCGAGCTTCCCATACTAACTAATTTC
40  Nacaeus_longulus
    ---------ATTCTATCTTACCCGGGTTTGGTTTAATTTCTCATATTATCAGCCAGGAAAGTGGGAAAAAGGAAACCTTTGGATCT
    CTAGGAATAATTTATGCTATATTAGCTAT---TGGATTCCTGGGATTTATTGTTTGGGCTCACCATATATTCACAGTAGGCATAGA
    CGTAGATACCCGAGCTTATTTTACATCAGCCACAATAATTATTGCAGTTCCGACCGGAATTAAAATTTTTAGATGACTAGCCACAC
    TTCATGGCAC------ACAAATTAATTATTCGCCGTCTATCCTCTGAGCTCTAGGTTTTGTATTTTTATT---------TACAGTA
    GGTGGACTAACAGGAGTAATTCTAGCCAATT------CTTCCATTGATATTATCTTACACGATACTTATTACGTAG--TAGCTCAT
    TTTCATTATGTTTTATCAATAGGAGCTGTTTTCGCTATTATAAGAGGTTTCATTCACTGATTTCCCCTATTTACTGGGCTCTCTCT
    CAAGGAAAAAATATTAAAAATTCAATTCATAATTATATTTATTGGAGTAAATTTAACATTCTTTCCCCAACATTTTCTGGGGCTTG
    CAGGTATACCACGACGATACTCAGATTACCCCGACGCATACACCCCTTGAAATGTAATTTCCTCGATTGGGTCTATTATATCTATA
    ATTGGAATTTTATTTATAATTTTTATCATCTGAGAAGCTTTTTCTGTCCAACGAAAGAGGATTTCCACTCTTAATTTTTCATCCTC
    AATTGAATGAAACCACTACATACCCCCGTCTGAACACACTTATTCCGAGCTTCCCATACTAACTAATTTC
41  Osorius_freyi
    --------ATTTTGATTCTTCCAGGATTTGGTTTAATTTCTCATATTGTTTGTCAAGAAAGAGGAAAAAAGAAACATTTGGTTCA
    CTAGGTATAATTTATGCTATACTATCAAT---TGGTTTATTAGGTTTTATTGTTTGAGCACATCATATATTTACAGTTGGAATAGA
    TATTGATACACGAGCTTATTTTACTTCGGCAACTATAATTATTGCTATTCCTACTGGCATTAAAATTTTTAGTTGGTTAGCAACTT
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     TATATGGATC------TCAAATCACTTTTAATCCTTCTATTTTATGAGCTTTAGGATTTATTTTTTTATT---------TACTGTA
     GGAGGTTTAACGGGGATTATTTTAGCAAATT------CATCTATTGATATTGCTTTACATGATACTTATTATGTTG--TTGCTCAT
     TTTCATTATGTTTTATCTATAGGGGCAGTATTTGCTATTTTTGGAGGATTTATTCATTGATATCCTCTCTTTACTGGTCTTACTTT
     AAATAATTTTTATATAAAAATTCAATTTTTTTCTATATTTATTGGAGTTAATTTAACATTTTTTCCTCAGCATTTCTTAGGATTAG
     CTGGGATACCTCGTCGATATTCTGATTATCCTGATTATTTTTTAACTTGAAATATTATTTCTTCTTTAGGTTCAATTATTTCTTCA
     ATAAGAGTTTTTCTATTTTTATATATAATTTGAGAAAGTATAAATTTATCACGAAAAATATATACTCATCTCAATTTAACTACTAA
     TATTGAATGATATCAACTTTCTCCTCCTGTAGAACATAGATATTCAGAATTACCTATTAATATTTACTTC
42   Plastus_sp.
     -----TATATCTTAATTTTACCAGGATTCGGACTCATCTCCCATATTATTAGACAAGAAAGAGGAAAAAAAGAAACATTTGGAACT
     TTAGGAATAATTTATGCAATAATAGCAAT---TGGATTACTAGGATTTGTAGTATGAGCACATCATATATTTACAGTAGGTATAGA
     TGTAGATACCCGAGCTTATTTTACTTCTGCTACAATAATTATTGCCGTTCCCACAGGAATTAAAATTTTTAGATGACTAGCCACCC
     TTCACGGATC------CCAAATTTCATACTCACCATCCCTATTATGAGCATTAGGATTTGTATTTCTTTT---------TACTATG
     GGAGGATTAACAGGTATTATTTTAGCTAATT------CTTCAATTGATATTATTCTACATGATACATACTATGTAG--TTGCTCAT
     TTTCACTACGTCTTATCAATAGGTGCTGTATTTGCAATTATAGCTGGAATTATTCAATGATTTCCATTAATAACTGGATTAACCTT
     TAATGAAAAACTTTTAAAAATTCAATTTATCTCCATATTCATTGGAGTAAATATAACATTTTTCCCTCAACATTTTTTAGGATTAG
     CAGGAATACCACGACGATACTCTGATTACCCGGATGCCTTCTATACTTGAAACGTAATTTCATCAATTGGATCAATTATTTCAATA
     ATCAGAATTTTATTCATAATTTATATCACATGAGATATATTCTTCTCTAACCGAAAAAGAATTTCCAACTTAAATTTTTCTTCCTC
     TATTGAATGATTTCAAAAAACACCTCCTGCAGAACATAGATACTCTGAATTACCAATAATTTCTTTATTC
43   Renardia_sp.
     ------------------TT-----------------------TATTTTTGGGGCTTGAGCCGGAATAGTAGGAACTTCTTTAAGA
     ATAT---TAATTCGAGCAGAACTAGGTAATCCTGGAACTTTAATTGGAGATGATCAAATCTACAATGTAATTGTTACAG---CTCA
     TGCTTTTGTGATAATTTTTTTTATAGTTATACCTATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATACTTGGAGCAC
     CTGATATAGCTTTCCCTCGAATAAATAATATAAGATTTTGACTTTTGCCTCCTTCACTTACTTTACTTTTAATAAGAAGAATAGTA
     GAAAGAGGAGCTGGAACTGGATGAACAGTTTATCCACCTCTCGCAGCTAATATTGCTCATAGAGGTTCTTCAGTAGATTTAGCAAT
     TTTTAGTCTTCATT-----TAGCTGGAATTTCCTCTATTTTAGGGGCTGTAAATTTTATTACTACAATTATCAATATACGATCAAT
     TGGAATAACATTTGATCGAATACCTTTATTTGTATGATCAGTAGCTATTACAGCCTTATTACTTCTCTTATCATTACCCGTATTAG
     CTGGGG--CTATTACTATACTTTTAACTGATCGAAATTTAAATACCTCTTTTTTTGACCCAGCAGGAGGGGGTGACCCAATTCTAT
     ACCAACATTTATTT-----------------------------------------------------------------------
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44   Eupiestus_sp.
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45   Piestus_extimus
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46   Siagonium_americanum
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48   Philonthus_sp.
     --------------CTTACCGCCGTTCCGCGTGATCGGCGACAACCTGAAGGACCGCTTCGACGGCGCTTCCCGCGTGATGCTGAG
     CAA-CGCGGCCAACGGAAGGAACACCAACCTAGCGCACAACGGCCGCCCGAAACAAGACAAGCTCTCCAACAACATCGCCTCCAAC
     AGCATCCACAGCAAACGCGAGAACCGACCCAGGAAGTACAAATACGGCTTCCAGCTGAAACCCTACAATCCCGACCATAAGCCCCC
     TAGTCCTAAGGATTTGGTCTATTTGGAACCCTCGCCCGGTTTCTGCGAGAGGAACCCGAAGTTGGGGATTCAGGGTACGCACGGGA
     GGCAGTGCAACGACACGTCGATCGGTGTGGATGGTTGCGATTTG-----------------------------------------
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49   Aleochara_sp.
     ACTTGCTGGATGCGGCTGCCGAACTTCCGCGTGATTGGCGATAACCTGAAGGACCGCTTCGACGGCGCCTCGCGCGTAATGGTGTC
     GAA-TACGGGCGCGCGCAGCAACGC------------CGCGCAGCGTCCGAAGGCGGACAAGATCAGCAACAGCATCACGTCGAAT
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    AGCATTCACAGTAAGCGCGAGAACAAGCCGCGCAAGTACAAGTACGGCTTCCAGCTGAAGCCGTTCAATCCGGACCACAAGCCGCC
    GAGCAGCAAGGACCTCGTCTACCTGGAGCCGTCGCCCGGCTTCTGCGAGAAGAACCCGAAGCTGGGCATCCAGGGCACGCACGGCC
    GCCAGTGCAACGAGACGTCGATCGGCGTCGACGGCTGCGACCTGATGTGCTGCGGCCGCGGCTAC--------------------
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50  Scaphium_castanipes
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51  Scaphidium_sp.
    ------------CGCCTTCCTCCTTTCCGAGTGATCGGCGACAACCTGAAGGACCGCTTCGACGGTGCATCTCGCGTCATGATCAG
    CAA-CACGGGCAACTCGAGGAACGCGAACAA--TGC-CGGTGGACGATCCAACCAGGACAAACTTTCCAACAGCATCGCCTCCAAC
    AGCATCCACAGCAAGCGCGAAAACAAACCCAGGAAGCACAAGTACGGTTTCCAGCTGAAACCATTGAATCCCGACCACAAACCACC
    AACGGTTAAGGATTTGGTTTACTTAGAACCGTCACCTGTGTTCTGCGATAAGAATCCAAAGTTAGGTATACAAGGCACGCACGGTA
    GGCAGTGCAATGATACTTCCAGAGGTTTGGATGGCTGTGATTTGATGTGTTGTGGAAGAGGGTACAGGCGGCAGGAAGTGGTAGTG
    GTTGAGAGATGTAACTGTACT
52  Scaphisoma_sp.
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53  Bledius_sp.
    ACTTGCTGGATGCGGCTCCCACCATTCCGCGTCATCGGCGACAACCTAAAGGACCGCTTCGACGGTGCCTCGCGCGTCATGCTCAG
    CAA-CGCGGGCAACTCGCGCAACGCCAACAG--CGC-CCCAGGCCGCCCCAAGCAGGACAAACTCTCCAACAGCATAGCTTCAAAC
    AGCATCCACAGCAAGCGCGAGAACAGGCCGCGCAAGTACAAGTACGGCTTCCAGCTGAAACCGTACAACCCCGACCACAAACCGCC
    CAGCGCCAAGGACCTGGTGTACCTGGAGCCCTCGCCCGGCTTCTGCGAGCGCAACCCCAAGCTGGGCATCCAGGGCACGCACGGGC
    GCCAGTGCAACGACACGTCGATCGGCGTCGACGGCTGCGACCTGATGTGCTGCGGCCGCGGCTAC--------------------
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54  Carpelimus_sp.
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55  Ochthephilus_sp.
    AACTGCTGGATGCGGCCGCCGCCTTTCCGCGTCATCGGGGATAATCTGAAGGATCGGTTCGACGGGGCCTCTCGGGTCATGGTCCA
    GAATCGGCGGCAA------CAGTGCACGCAA-------CTGAATGGTCCCAAACAGGACAAACTCTCCAACAGCATCAGTTGCAAT
    AGCATCCCAATAAAGCGTGAGAACAAGCCGCGAAAATACAAATACGGCTTCCAGTTGAAGCCGTACAACTGGGACCACAAACCGCC
    GAGCAGCAAGGACTTTGTTTACCTGGAGCCGTGGCCGGGTTTTTGCGAGAGAAACCCGAAGTTAGGGATCCAGGGCACGCACGGCG
    GCCAGTGCAATGACACCTCCATGGGAGTAGACGGGTGCGATCTGATGTGGTGCGGTAGAGGCTAC--------------------
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56  Oxypius_peckorum
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57  Oxytelus_bengalensis
    AAATTCTGGATGCGGCCGCCGCCCTTCCGCGTGATTGGGGATAACCTGAAGGACCGGTTCGACGGCGCCTCTCGCGTAATGGTCCG
    -AA-TACGGGCGCGCGCAGCAACGAC----------GTGCAGTCGTTCGAAAGAGGACAAGATTAGCAACAGCATCACGTCGAAT
    AGCATTCCCAGTAAGCGGGAGAACAAGCCCCGCAAGTACAAATACGGGTTTCAGCTGAAACCGTACAATCTGGACCACAAGCCGCC
    GAGCAGCAAGGACTTTGTTTACCTGGAGCCGTCGCCCGGGTTTTGCGAGAAGAACCCGAAGCTGGGCATTCAGGGCACGCACGGCC
    GCCCATGCAACGAGACGTCGATCGGCGTAGACGGGTGCGACTTGATGTGCTGCGGGCGCGGCTAC--------------------
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58  Homalotrichus_substriatus
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59  Eleusis_sp.
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60  Lispinus_sp.
    ACTTGCTGGATGCGCCTGCCGCCCTTCCGCGTCATCGGCGACATCCTCAAGGATCGCTTCGATGGCGCCTCGCGCGTCATGGCCAA
    CAA-CGGCGGCAA------CAGTGCGCGCAA------CTCCAATCGTCCCAAACAGGACAAACTCTCCAACAGCATCAGCTCCAAT
    AGCATCCACTCAAAGCGTGAGAACAAGCCGCGAAAATACAAATACGGCTTCCAGTTGAAGCCGTACAACTCCGACCAGAAGCCGCC
    TAGTAGTCGCGATCTGGTTTACTGGGAGCCGTCGCCTGGCTTCTGCGAGCGCAACACGAAGCTAGGGATACCGGGCACCCATGGCC
    GCCAGTGTAATGACACCTCCATGGGAGTGGACGGGTGCGATCTGATGTGCTGCGGAAGAGGGTAC----------------------
    ----------------------
61  Nacaeus_longulus
    ACTTGCTGGATGCGCCTGCCGCCCTTCCGCGTCATCGGCGACATCCTCAAGGATCGCTTCGATGGCGCCTCGCGCGTCATGGCCAA
    CAA-CGGCGGCAA------CAGTGCGCGCAA------CTCCAATCGTCCCAAACAGGACAAACTCTCCAACAGCATCAGCTCCAAT
    AGCATCCACTCAAAGCGTGAAAACAAGCCGCGAAAATACAAATACGGTTTCCAGTTGAAGCCGTACAATTCCGACCAGAAGCCGCC
    TAGTAGCAAGGATCTGGTTTACTTGGAGCCGTCGCCTGGCTTCTGCGAGCGCAACACGAAGCTAGGGATACCGGGCACCCATGGCC
    GCCAGTGTAATGACACCTCCATGGGAGTGGATGGGTGCGATCTGATGTGCTGCGGAAGAGGGTAC----------------------
    ----------------------
62  Osorius_freyi
    ACTTGCTGGATGCGACTGCCGCCGTTCCGCGTGATCGGCGACAACTTGAAGGACCGTTTCGACGGCGCCTCCCGGGTCATGATCGG
    CAG-CAACGCGCC------CAATGCGGCCCG------CAGCAACCGCCAGATGCCAGACAAGCTCTCCAACAGCATCAGCACGAAC
    AGCATCCACAGTAAGCGCGAGAACAAGCCGCGAAAGTACAAGTACGGGTTCCAATTGAAGCCGTACAATCCCGATCACAAGCCGCC
    CAGCGTCAAGGATTTCGTCTATTCCGAACAATCGCCAGGGTTCTGCGAGAAGAACGCGAAGTTGGGCATACCAGGCACGCACGGCC
    GGATGTGCAAAGACACGTCCATCGGGGTGGACGGGTGCGATCTGATGTGCTGCTGTCGCGGCTAT----------------------
    ----------------------
63  Plastus_sp.
    ----------------CGTCGCGATTCCGCGTGATCGGCGACAACCTGAAGGACCGCTTCGACGGCGCGTCGCGCGTCATGGTCGG
    CAA-CAGCGGGAA------CAACGCGCGCAG---------CGGCCGCAGCAAGACGGACAAGCTGTCGAACAGCATCTCGTCGAAC
    AGCATACACAGCAAGCGGGAGAACAGGCCGCGGAAGTACAAGTACGGCTTCCAGCTGAAGCCGTACAATCCGGAGCACAAGCCGCC
    CAGCCCGAAGGACCTCGTCTACCTGGAGGTGTCGCCCGCCTTCTGCGAGCGGAACACGAAGCTGGGCATACCCGGCACGCACGGCC
    GCCACTGCAACGAGTCGTCGATCGGCCTCGACGGCTGCGACCTGATGTGCTGCGGCCGCGGCTAC----------------------
    ----------------------
64  Renardia_sp.
    --------------GCTACCGCCCTTCCGGGTAATCGGTGACAATCTGAAGGAGCGATTCAATGGCGCTTCACGCGTCATAGCCAG
    CTA-TGGTGGTGGAAACAATCAGCCGCGTAA--CCA-TCACAATCGGCAGAARGTAGACAAACTGGCGAAYAGCATCTCATCGAAY
    AGTATCCACAGTAAGCGCGATAGTAAGCCGCGCAAATACAAGTATGGTTTTCAGTTGAARCCATACAATCCRGAATTCAAGCCGCC
    AACCGTTAAAGACTTGGTGTATTTGGAACCKTCGCCTGGATTCTGTGAACGGAATACTAAGCTGGGTATTCAGGGTACATACGGTA
    GGGAGTGTAACGATCAGTCGGGAGGTTTAGACGGTTGTGGGTTGATGTGTTGCGGACGGGGCTATCAACGACAGGAAATCACGGTG
    GTGGAGCGGTGCAATTGCACA
65  Eupiestus_sp.
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66  Piestus_extimus
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67  Siagonium_americanum
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68  ;
69  end;
70
71  begin mrbayes;
72  charset 28S = 1-1636;
73  charset COI = 1637-2480;
74  charset Wg = 2481-2931;
75  partition Names = 3: 28S, COI, Wg;
76  end;
77
78  begin mrbayes;
79  set partition=Names;
80      lset applyto=(1,3) nst=6 rates=invgamma;
81      lset applyto=(2) nst=6 rates=gamma;
82  end;
83  Begin mrbayes;
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84   lset nst=1 rates=gamma coding=variable;
85   mcmc ngen=10000000 samplefreq=100 printfreq=100 nruns=2 nchains=4 mcmcdiagn=yes
     diagnfreq=100 savebrlens=yes relburnin=yes burninfrac=0.25;
86   END;
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APPENDIX 9 – Merged data matrix of morphological characters used for the phylogenetic analysis of the subfamily Piestinae (Coleoptera: Staphylinidae).